EFFECTS OF CALCIUM AND PH STATUS ON SUBSOIL ROOT DEVELOPMENT OF LEGUMES IN AN ALUMINUM RICH SOIL

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INTRODUCTION

Most legumes generally grow poorly in soils that are too acidic. Those that grow give extremely low fodder yields in terms of tons/acre. On close examination it has been found that such plants have a very poorly developed root system. Shallow rooting generally renders the plants ineffective in absorbing the profile-stored moisture and nutrients needed for maximum growth and yields. Poor root development has previously been attributed to such factors as unfavorable soil, physical conditions, H-ion toxicity, Ca deficiency and subsoil acidity which result in toxic concentrations of certain elements. It has been established (Magistad, 1925) that over a certain pH range (acidity greater than pH 5.0) it is the presence of Al in the ionic form which is the primary limiting factor for susceptible species. More recent evidence indicates that Al toxicity limits the penetration of legume roots into an acid subsoil (Schmehl, et al., 1950; Wright and Donahue, 1952; and Adams and Lund, 1966). Root growth of young seedlings are particularly susceptible to injury by toxic concentrations of Aluminum. The failure to initiate roots occurs during the phase of early seedling development when the seedling's nutrient source is shifting from its own seed reserves to active uptake from the external medium. Therefore forage legumes with small seed reserves are particularly susceptible to the toxic effects of the aluminum ion.

Lime applications in addition to supplying Ca also reduces soil reaction. Calcium rapidly modifies the soil reaction in the top soil, but only slowly and less ectensively alters reactions in the subsoil. Subsoils play a significant role in plant growth simply because of their

effect on root development. An adequate root system in the subsoil would undoubtedly afford the plant with a larger amount of nutrients and an additional volume of soil water especially in years when water is limiting.

The purpose of this study therefore was to determine the extent to which Ca levels and/or pH adjustment affected the development of that portion of the root system growing in an unfavorable acid subsoil zone.

LITERATURE REVIEW

CALCIUM NUTRITION

The beneficial effect of Ca supply and root development (particularly subroot) is a well known phenomenon (Haynes and Robbins, 1948; Burstrom, 1952; Ekdahl, 1957 and Foy and Brown, 1964). In the absence of Ca from the ambient medium, root growth was seriously arrested both at the apex and in the development of lateral primordia. As the Ca translocation within the root tissue to the development root apex is low (Weibe and Kramer, 1954), Ca must be present constantly in the surrounding medium for normal root growth to develop (Haynes and Robbins, 1948). Root hair development was also considerably influenced by calcium. According to Ekdahl (1957) low amounts of Ca resulted in short, irregularly shaped hairs. Burstrom (1964) has shown that low supplies of Ca retarded elongation of pea stems by restricting cell elongation.

Work by Biddulph, et al. (1958) with bean plants indicated that after Ca was deposited in roots or foliage it was not circulated. Gauch (1940) and Jackson and Evans (1962) reported that due to low cotyledon contents and sluggish movement of Ca from the cotyledon resulted in an early onset of severe Ca deficiencies in developing leaves and roots of young soybean seedlings.

Andrew and Norris (1961) showed that without added Ca, tropical legumes did not show any visual symptoms of Ca deficiency and their yields increased when higher amounts of $CaCO_3$ were added. Temperate legumes however under the same conditions showed visual symptoms of Ca deficiency. Stylosanthes bojeri Vog. and Trifolium repens L. are the

legumes noted to be very efficient in Ca-extraction whereas Medicago sativa L. was a highly inefficient specie.

They concluded that tropical legumes tend to be more efficient than the temperate species at Ca-extraction.

Loneragan, et al. (1968) reported from solution culture experiment that some legumes grew much better at low concentrations of Ca (2.5 µm to 10 µm/liter) than many gramineae. The minimal concentration required to produce maximum growth of plants and eliminate Ca deficiency symptoms varied from (2.5 µm--1000 µm/liter). Crops such as the legumes, particularly alfalfa and clover, require considerably more Ca and Mg than most other crops and consequently remove large amounts of bases. An average crop of alfalfa will remove about 100 lbs of Ca per acre, clover about 60 lbs and soybean 30 lbs.

The effect of other ions in the medium on the uptake of Ca by root tissue was often quite large (Epstein, 1961 and Johnson and Jackson, 1964). The presence of Al in ambient medium has a strong depressing effect on Ca uptake by the root. Therefore, Al injury has been associated with decreased uptake and utilization of Ca by plants (Rorison, 1958; Foy and Brown, 1964 and Johnson and Jackson, 1964). Many of the Al toxicity symptoms, particularly on root growth were quite similar to Ca deficiency symptoms (Hallsworth and Greenwood, 1957 and Rorison, 1958).

Some success in overcoming Al toxicity by increasing Ca concentrations has been reported by Rios and Pearson (1964) and Clarkson (1965). Hallsworth, et al. (1957) found no beneficial effects of increasing Ca to quite a high levels were noted when Al

concentrations was in the order of 10--20 ppm. Millikan (1949) indicated a slight alleviation of Al toxicity by high Ca supplies.

THE EFFECTS OF ALUMINUM ON PHOSPHORUS UPTAKE

Aluminum injury similarly has also been associated with decreased uptake and utilization of phosphorus (Burgress, et al., 1923; Walliham, 1948; Wright, 1943; Wright, et al., 1953 and Foy, et al., 1964). It was observed that there was a reduction in the uptake of P in both the plant tops (Foy and Brown, 1964) and the roots with increasing Al concentration (Humphreys and Truman, 1964; Ragland and Coleman, 1962; Randall and Vose, 1963; MacLeod and Jackson, 1964). It was found that there was abundant inorganically bound phosphorus present in barley roots grown in contact with Al in soil culture and not in the roots from culture solutions led to suggest that there was a combination of PO_4 with Al in the plants (Wright, 1941; Wright and Donaghue, 1953).

Plant symptoms of phosphorus due to Al toxicity are similar to those of severe phosphorus deficiency. Corn and mustard showed a purple color which became more pronounced as Al concentration was increased. Barley and oats became chlorotic; leaves died back from the tips and the base of the plants became purple in color. The roots of Al sensitive plants, such as clover and barley, became stubby and coral-like with little or no side branching.

THE RELATIONSHIP BETWEEN SOIL ACIDITY AND AL SOLUBILITY

It has been reported that the shallow rooting of plants grown in acid soil was due to Al-ion toxicity (Magistad, 1925; Waterpaugh, 1936;

Rios, et al., 1964; Adams, et al., 1966). Dwarfing and root injury were found to be the first effects of Al toxicity (McLean, 1927). The roots develop a brownish coloration and the main roots failed to elongate rapidly, becoming thick, swollen and distorted. Lateral root development was inhibited with the laterals remaining as short abortive stubs.

The amount of Al remaining in solution is strongly affected by pH. At very low pH values Al is present almost entirely as free trivalent ions. Rorison (1958) noted that the trivalent Al-ion is unlikely to be in the soil solution if the pH is above 4.5. But as the pH of the soil solution increases, water molecules associated with the Al ion [Al $(H_2O)6$] are replaced by hydroxyl ion to form $Al(OH)_3 \cdot 3H_2O$, and the aluminum ions are gradually precipitated out as insoluble hydroxides. At pH values above 7.5, aluminum appears again in solution as aluminate (Al_2O_3) (Jones, 1961). Magistad (1925) reported a complete precipitation of Al in solution at pH levels between 5.8 and 7.0. Contrary to previous workers findings, Clark (1966) and MacLeod, et al., (1967) stated that there is no direct relation between pH and the concentration of soluble Al.

The availability of extractable Al was reported by Plucknett and Sherman (1963) to be influenced by rainfall. They found a positive correlation existed between extractable Al and rainfall periods. The percentage of Al saturation of soils was reported to increase with decreased drainage (Rorison, 1958).

THE EFFECTS OF ALUMINUM ON PLANT NUTRITION

Many workers have reported that excess Al causes plants to wilt and decreases the absorption of Ca, P, and K. The uptake of other elements such as Mn, Na, B and Fe were also affected by Al (unpublished work of Foy, 1963, as cited by Foy and Brown, 1964). MacLean and Gilbert (1927) found that Al reduced absorption of cationic dyes, nitrates and water by corn. Szues (1912) as quoted by Hutchinson (1942) stated that Al caused the protoplasm of cells to "gel", and thus reduced the overall permeability of roots (Magistad, 1925; MacLean, et al., 1927). Aluminum was also found to accumulate in the nuclei of cortical cells in corn and cabbage roots.

THE EFFECTS OF ALUMINUM ON PLANT GROWTH

Although the detrimental effect of small amounts of Al on root growth has been well documented (Gilbert, et al., 1935; Hortenstine, et al., 1961; Adams, et al., 1966; Waterpaugh, 1936), toxic levels of Al appear to be different for each soil. Inhibition of plant growth in an acid soil is proportional to the amount of exchangeable Al or the level of Al saturation (Ragland and Coleman, 1959). However, levels of exchangeable Al that are toxic in one soil may be well below the toxic level in another soil.

Plant species differ greatly in their tolerance to Al in acid soils containing high levels of soluble or exchangeable Al (Burgress, et al., 1923; Hartwell, et al., 1918; McLean, 1927; Ligon, et al., 1934). Lettuce, mustard and some other plants are very sensitive while corn, wheat and soybeans are tolerant. A concentration of soluble Al

as low as 1 ppm has been shown to restrict root growth in legume species such as alfalfa while 2 ppm inhibited root growth and caused severe symptoms of toxicity (MacLeod and Jackson, 1965). Kliewer (1891) found that 0.4 to 0.7 ppm Al in solution greatly reduced effective nodulation of birdsfoot frefoil and 1.5 ppm prevented root elongation. In soil the Al ion toxicity is manifested above 0.5 mg/l (Pfeffer, et al., 1933). This agrees with the work of Magistad (1925). Of the twenty-three plant species found growing on bauxite soils of Hawaii, 13 of them were Al accumulators, with more than one thousand parts per million of Al on a dry weight basis (Mooman, et al., 1959). Plants such as Lycopodium alpinum had as much as 27 % Al in its ash while only traces were found in other species of Lycopodium (Pfeffer, 1899). Jones (1961) and Foy, et al. (1967) related Al tolerance to the maintenance of P status in the plant. On the other hand, Orrellette and Dessureaux (1958) concluded that differential Al tolerance of alfalfa clones was not due to Al-P interaction. The nature of the differential tolerance is still very much a puzzle.

THE EFFECTS OF LIME APPLICATION ON ALUMINUM AND SOIL ACIDITY

Toxic concentrations of exchangeable Al can be reduced by the application of lime. Liming not only supplies Ca, but raises the pH of the soil, which in turn decreases Al, Mn and Fe solubility. This enhances the availability and plant uptake of elements such as molybdenum, phosphorus, calcium and magnesium. Kehoe and Curnow (1963) pointed out that greater uptake of P by plants from limed soil is due to improve ability of plants to take up P rather than to an increased

rate of supply by the soil. Lime response is generally associated with increased P uptake by plants; however, plant species differ markedly in their response to lime. Clements (1960, 1963) found that heavy application of lime is powdered coral stone resulted in increased phosphorus concentration in the plant and reduced soluble Al in the soils studied. An application of two tons of lime to a humic ferruginous latosol on the Island of Maui produced a substantial increase in the yield of forage and seed of Kaimi clover (Younge, 1959). Rios (1958) reported that roots found in unlimed subsoil were thick and poorly-branched as compared to the finely divided and well-branched root systems in the limed subsoil.

MATERIALS AND METHODS

I. MATERIALS

a) Soils

The soil used in this experiment was a Pauwela silty clay from the Kaupakaulua area of Maui, Hawaii near the site chosen for the modal profile description of this soil. The Pauwela soils are members of the Humic Ferruginous Latosol great soil group, and are classified as Tropohumults under the Seventh Approximation. They occur on the Island of Maui at elevations from sea-level to 1,500 feet where annual precipitation ranges from 80 to 150 inches. Soils of this group have low to moderate cation-exchange capacities. The cation-exchange capacity of the Ap horizon ranges from 2 to 30 meq per 100 g soil. The C.E.C. for the subsoil appeared to be slightly higher than the topsoil. The important secondary minerals of the Pauwela soil are goethite and gibbsite.

The topsoil or the Ap zone soil has a depth of 0-7 inches and consisted of a grayish-brown silty clay. It has a strong to medium granular structure, is very friable when moist and non-plastic when wet (Cline, 1955). In addition, it has a high bulk density and the soil reaction ranges from pH 4.5 to pH 5.5, but the soil that was used in this experiment had a pH of 4.6. Weed roots were very numerous.

The subsoil or the B_2 zone, from a depth of 9-30 inches is a yellowish-red silty clay. It has a moderately developed fine blocky structure, a moderate bulk density, and a pH of 4.8.

Few roots were present. It was observed in preliminary experiments that when carbonates or hydroxides of potassium were added to the

subsoil to raise pH without lime, de flocculation occurred as these compounds dispersed the soil particles. The soil structure became massive and dried slowly to a very hard mass.

b) Legumes

The four legume species were used as test crops are listed below:

- White clover (Trifolium repens L.) cv. Regal.
- Big Trefoil (Lotus uliginosus Schk.) cv. New Zealand 4703.
- Stylo (Stylosanthus gracilis) cv. "Schofield".
- Desmodium (Desmodium intortum Mill. Urb) cv. Hawaii 4331.

Of the four species, T. Repens and L. uliginosus are temperate zone legumes while S. gracilis and D. intortum are tropical legumes.

II. METHODS

a) Preparation of the soil

The top and subsoils were air-dried separately to remove excessive moisture. The larger aggregates were crushed and passed through a quarter-inch mesh sieve to remove foreign material, weed roots, and stones. The screened soils were stored in polyethylene bags to conserve moisture.

b) Determination of lime requirement

The lime requirement was determined from titration curves for the top and subsoils (Chapman and Pratt, 1961). Seven samples of 25 g (O.D. soil) each from the Ap zone and B_2 zone were placed in six-inch open-mouthed bottles.

Increments of $Ca(OH)_2$ were added to supply 0, 2, 4, 8, 12, 16 and 20 meCa/100 g to the samples. These amounts were equivalent to 0, 1, 2, 4, 6, 8 and 10 tons of $CaCO_3$ per acre. Distilled water was added to give a soil : water ratio of 1:2 and the samples allowed to equilibrate with occasional shaking, till constant pH was reached. The pH was measured in the soil suspension with a Beckman Zeromatic pH meter. A titration curve of pH vs me $Ca(OH)_2$/100 g soil was drawn for both the top and subsoil (Fig. 1 and Fig. 2).

c) Determination of exchangeable Al at varying soil pH

Exchangeable Al at varying soil pH was determined by extraction with N KCl. The weighed soil samples (10 g O.D.) obtained from determination of lime requirement were placed in 100 ml Erlenmeyer flask and 50 ml 1 N KCl was added to each. The samples were shaken on a mechanical shaker for 30 minutes at low speed and then allowed to equilibrate overnight. They were then filtered and washed four times using a total of 50 ml extracting solution. The filtrate was made to 100 ml volume with 1 N KCl solution.

Aluminum in the filtrate was determined by the Aluminon method described by Chapman and Pratt (1961). A 2 ml aliquot of filtrate was transferred to a 50 ml volumetric flask, diluted with about 10-15 ml distilled water, and mixed with 2 ml 1:100 thioglycolic acid to remove Fe interference. Ten ml of aluminon reagent were added and the pH was adjusted to 4.2 with 1:1 NH_4OH or HCl. The solution was diluted to almost 40 ml with distilled water and then heated in a boiling water-bath for exactly 16 minutes. After the solution had cooled to room-temperature, it was made to volume with distilled water, and the color

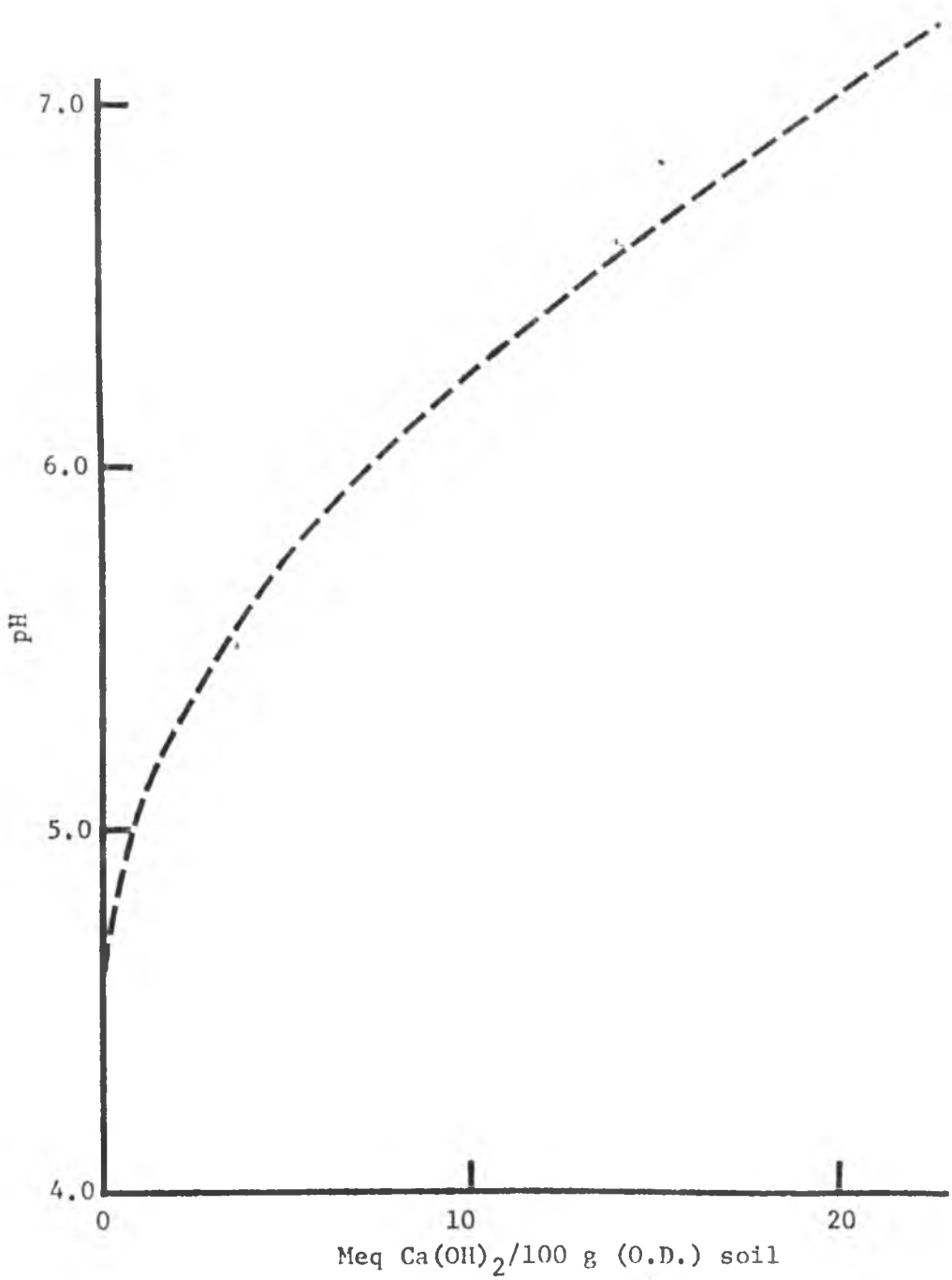


FIG. 1. TITRATION CURVE OF PAUWELA TOP SOIL (AP ZONE)

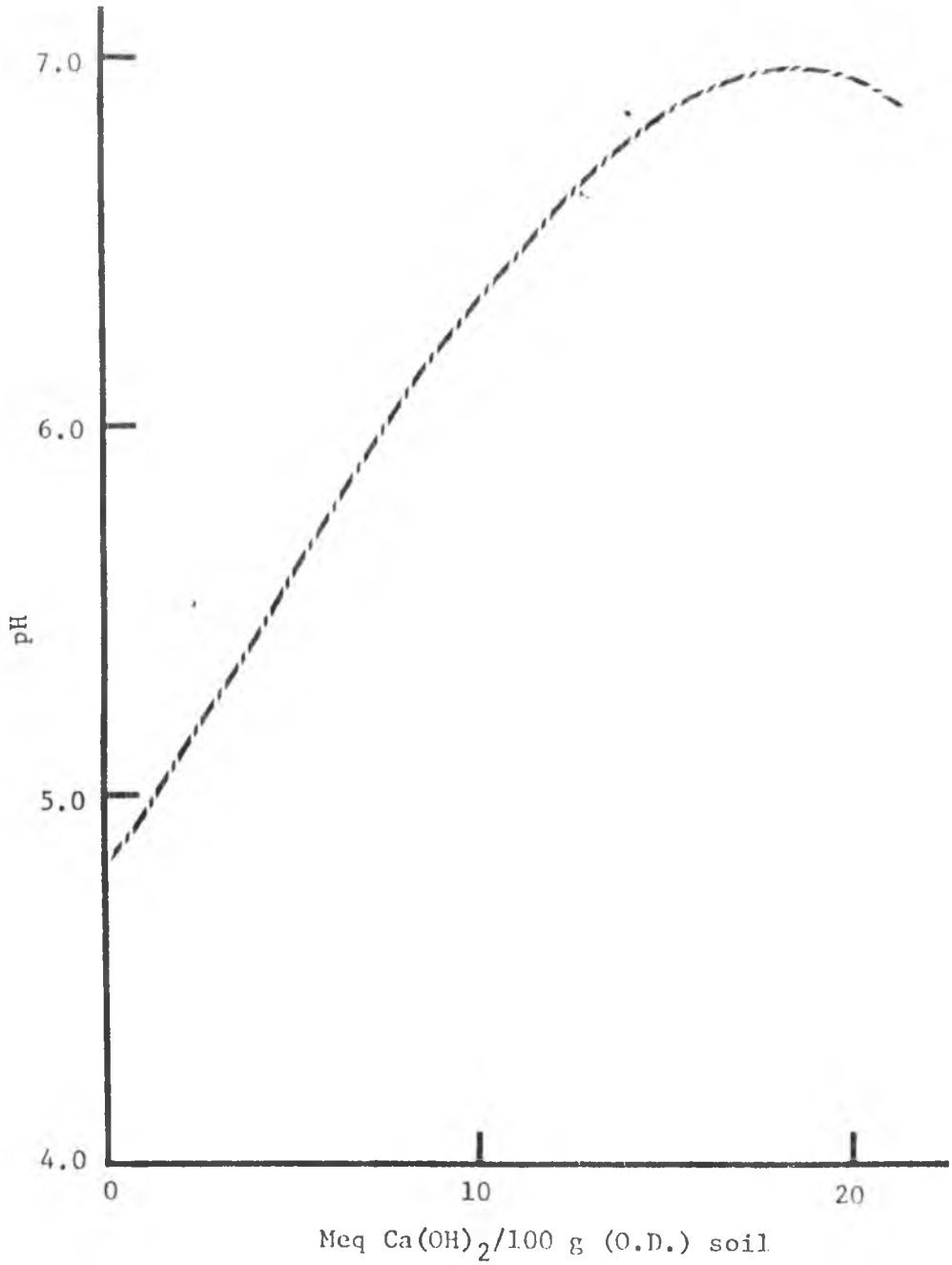


FIG. 2. TITRATION CURVE OF PAUWELA SUBSOIL (B_2 ZONE)

intensity read on a Coleman Junior colorimeter at 537.5 mμ. The concentration of Al in ppm was calculated from a standard curve.

d) Determination of C.E.C. and exchangeable Ca, Mg and K

Cation exchange capacity was determined using NH_4OAc pH 7.0 as described by Chapman and Pratt (1961). A soil sample weighing 10 grams was shaken with 200 ml of NH_4OAc for one hour and left standing overnight. It was then filtered and further leached with four 50 ml of extracting solution. The filtrate was made to a 500 ml volume and kept for the determination of exchangeable bases.

The leached soil sample was then washed three times with 50 ml methyl alcohol to remove excess NH_4OAc.

The washed soil plus filter paper was then placed in a 500 ml Erlenmeyer flask and shaken on an oscillating shaker for 30 minutes with 200 ml 4 % KCl. The mixture was filtered and washed with another 150 ml of KCl. The leachate was then transferred to an 800 ml Kjeldahl flask and the volume made to about 400 ml. After 10 ml of 1:1 NaOH and a few pieces of mossy zinc were added, the mixture was distilled into 50 ml of a mixture of 4 % boric acid and methyl red and Bromocresol green.

The boric acid was then titrated with standard H_2SO_4 and the C.E.C. calculated.

The exchangeable bases Ca, Mg and K were determined with the Perkin Elmer model 303 atomic absorption spectrophotometer directly in the ammonium acetate extracts. All standards were prepared with ammonium acetate.

e) Design and layout of pot experiment

This experiment consisted of three Ca levels, three pH levels, four species and three replications. The 3 x 3 x 4 factorial arrangement of treatments was laid out in a randomized complete block design. Three tables, each (35" x 106") were located in the green house and each table served as a replicate.

Each complete replication consisted of 40 cans (36 factorial treatments plus 4 controls) which were randomly arranged within the replicate. Particular attention was given to spacing the cans so that no mutual shading occurred.

f) Procedure

The experiment was started on October 31, 1969 in the former P.R.I. green house using 400 and 1,000 (O.D. Soil) of top and subsoil, respectively in each treatment. The topsoil was placed in 16 oz, Dixie (NO 2186) squat containers. A blanket application of nutrients was added to each container of the topsoil as follows:

500	ppm	P	as	H_3PO_4
100	ppm	K	as	KCl
10	ppm	Mg	as	$MgSO_4 \cdot 7\ H_2O$
10	ppm	Zn	as	$ZnSO_4 \cdot 7\ H_2O$
2	ppm	Cu	as	$CuSO_4 \cdot 5\ H_2O$
1	ppm	B	as	$Na_2B_4O_7 \cdot 10\ H_2O$
0.5	ppm	Mo	as	$(NH_4)6\ MO_7O_{24} \cdot 4\ H_2O$

Lime in the form of $CaCO_3$ was added to each pot to bring the pH to 6.5. The treated soil was watered to field capacity and allowed to

equilibrate for a period of two weeks. The pH of all the pots was checked at the end of this period.

Seeds of the test legumes were sown directly into the topsoil with the aid of a pair of forceps. Seeds of desmodium and stylo were scarified by soaking them in a small quantity (about 30 ml) of concentrated H_2SO_4 for 5 to 10 seconds to enhance germination. The treated seeds were washed and rinsed immediately 3 to 4 times with an excess of distilled water.

About five seedlings were grown in each pot. After a period of three to four weeks, the base of the pot was removed and the pot was superimposed onto a 46 oz can (4" diam. x 7" tall dimension, #5 tall) lined with a polyethylene bag and containing the treated subsoil. The top and subsoils were separated by a waxed nylon screen. The waxed nylon screen was prepared by dipping a 6" square of nylon screen into liquid wax (10 ml mineral oil to 3/4 lb parawax). This screen prevented leachate from the topsoil getting into the subsoil below and yet allowed roots to penetrate into the subsoil.

Watering of the subsoil was done through a 6" piece of rubber tubing (1/4" i.d.) inserted into a hole drilled about three fourths of an inch below the can rim such that about two and one half inches of the tubing was inside the can and three and one half inches was outside the can. The inner portion of tubing was sloped downwards at an angle of approximately 65° which permitted the tube to discharge at a point about one and one half inches below the center of the waxed screen (Fig. 3). Water was added through the tube to maintain subsoil moisture at near field capacity.

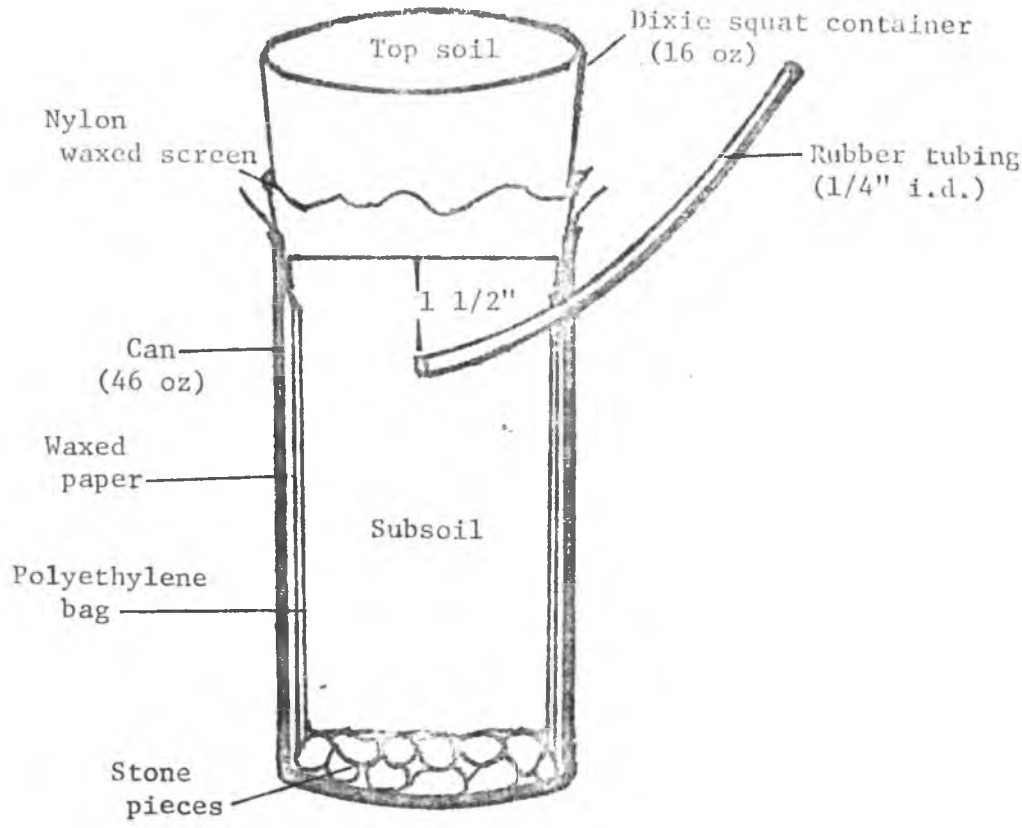
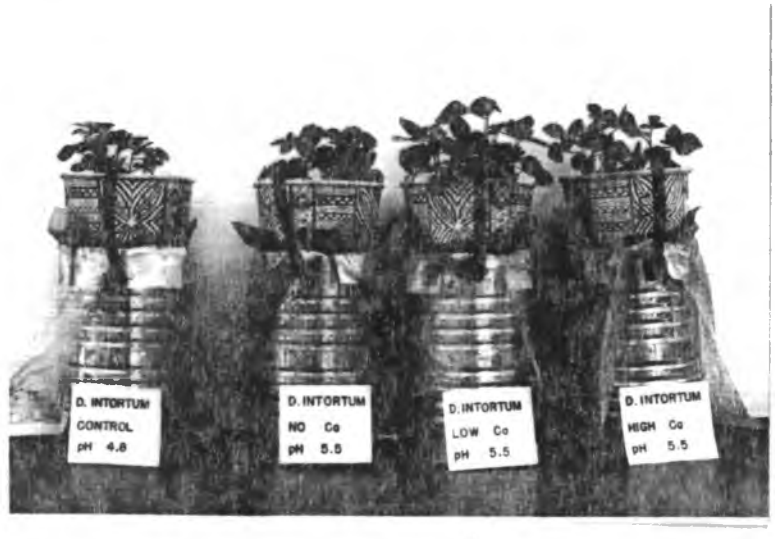


Diagram of pot setup



Photograph of pot set-up

FIG. 3. SET-UP FOR THE STUDY OF SUBSOIL ROOTS

The space between the inside of the can and the polyethylene bag was lined with waxed paper to prevent the polyethylene bag from adhering to the wall of the can. A layer of smooth gravel below the bag prevented it from adhering to the bottom of the can. Drainage holes were also provided in both the polyethylene bag and the base of the can.

Treatments in the subsoil consisted exclusively of three levels of Ca and three levels of pH. As the objective of this study was to determine whether subsoil root growth was dependent on Ca and/or pH, no nutrient whatsoever was further added to the subsoil.

The three Ca levels, viz. zero Ca, low Ca and high Ca, consisted of 0, 4.5 and 11.0 me Ca/100 g soil respectively. The three levels of pH at each Ca level were adjusted by using equivalent amount of Mg CO_3 or $CaCO_3$ or $CaSO_4$ to give pH 4.8, 5.5 and 6.3 as shown in Table I.

In the zero Ca series equivalent amounts of $MgCO_3$ were used to attain pH levels of 5.5 and 6.3 and the Mg content was equalized with $MgSO_4$. In the low Ca series, the level of Ca was equivalent to the amount of $CaCO_3$ required to each a pH of 5.5. The high pH treatments were attained with $MgCO_3$, $CaCO_3$, $CaSO_4$, and $MgSO_4$ in amounts necessary to equalize the mineral levels. For the high Ca series, the amount of Ca applied was determined by the amount of $CaCO_3$ needed to attain a pH of 6.3. The pH 5.5 level was attained by using $CaCO_3$ and $CaSO_4$. The mineral levels in all of the treatments was equalized with $MgSO_4$ (Table I). The treated soils were watered to field capacity and allowed to equilibrate for two weeks. The pH was checked individually at the end of this period.

TABLE I. CALCIUM AND MAGNESIUM SALTS ADDED TO ADJUST THE PAUWELA SUBSOIL TO THE INDICATED CALCIUM LEVELS AND pH VALUES

Treatment		Compounds applied (ppm)				Cations added meg/100 g soil	
Calcium level	pH value	$CaCO_3$	$CaSO_4$	$MgCO_3$	$MgSO_4$	Ca	Mg
	4.8	--	--	--	13,557	0	11.0
Zero	5.5	--	--	2,113	5,546	0	11.0
	6.3	--	--	5,160	--	0	11.0
	4.8	--	3,060	--	13,557	4.5	11.0
Low	5.5	2,250	--	--	13,557	4.5	11.0
	6.3	2,250	--	3,050	8,000	4.5	11.0
	4.8	--	7,480	--	13,557	11.0	11.0
High	5.5	2,250	4,420	--	13,557	11.0	11.0
	6.3	5,500	--	--	13,557	11.0	11.0

The top and subsoils were watered separately. Generally, two applications of 20-30 ml of distilled water were applied to the topsoil each day and 20-25 ml were added every second day to the subsoil. The amount of water added varied by about ± 8 ml. Water was applied to the topsoil with a plastic sprinkler bottle and to the subsoil with a 250 ml burette. Adequacy of watering was checked by means of index cans which were weighed regularly. The loss of water in the index cans served as a guide to the proper watering of the treated cans.

Since the legumes were not inoculated with rhizobium, the N requirement of the plants was met by supplying NH_4NO_3.

A total of 130 ppm N was applied to each pot in four split applications of 30, 40, 30 and 30 ppm given at two, five, seven and nine weeks after planting, respectively.

g) Harvesting

The plants were harvested on January 26, 1970, about eight weeks after pots were placed over the subsoil (or 85 days total growing period). The legumes (tops) were cut at ground level. The can was removed and the polyethylene bag cut to expose the subsoil which was then washed. Washing of the soils were done separately at the boundary of the two soil zones. The subsoil root was measured with a scale, before cutting, to determine the root length. The roots in each horizon and the tops were washed thoroughly.

The roots and tops were held separately in nylon netting and washed in a 0.01% detergent solution. The roots were then rinsed sequentially in four plastic containers, each containing about 500 ml

de-ionized water. The water was changed frequently. The washed roots and tops were bagged, dried at 70°C and weighed. The samples were ground in Wiley mill to pass a 40 mesh screen.

Separate 150 samples of topsoil and subsoil were collected from each treatment. A portion (about 20 grams) of each sample was used for pH measurement in a 1:1 soil : water mixture. The remaining soil was kept for other analyses.

h) Analytical methods for plant samples

A) Digestion of plant samples

A subsample (0.5 g) of the ground plant material was weighed into a micro-Kjeldahl flask to which 15 ml of 2:1 nitric-perchloric acid mixture was added, and allowed to stand overnight.

The samples were then heated and digested to the white fuming stage at which 3 to 5 ml of the colorless perchloric acid was left. The digested samples were diluted, transferred to a 50 ml volumetric flask and made to volume with distilled water. The blank samples were also prepared in the same way. For samples weighing less than 0.1 g, 10 ml (instead of 15 ml) of 2:1 nitric-perchloric acid mixture were used and the final volume of the digest was made to 25 ml.

B) Phosphorus determination

The method for P determination was similar to that described by Chapman and Pratt (1961). A 5 ml aliquot of the nitric-perchloric digest was transferred to a 50 ml volumetric flask and diluted with about 25 ml distilled water. Then 5 ml of Barton's

solution was added, the volume made to 50 ml with distilled water and mixed thoroughly. Color was allowed to develop for 30 minutes, then color intensity was read on the Coleman Junior colorimeter at 430 mμ. The concentration of P was calculated from a standard curve.

C) Aluminum determination

Plant aluminum was determined by the aluminon method as described by Chapman and Pratt (1961). The size of the aliquot varied from 1-5 ml depending on the plant part. The procedure was the same as that described previously for soil Al.

D) Calcium, Magnesium, Manganese and Potassium determinations

These elements were determined with a Perkin-Elmer model 303, atomic absorption spectrophotometer. The concentration of each element was calculated with reference to the standard curve for that element.

i) Analytical methods for soil samples

A) Exchangeable aluminum determination

The subsoil samples obtained at the end of the experiment were treated with a measured quantity of 1 N KCl, and a convenient aliquot was collected and analyzed for exchangeable aluminum. The method of extraction and aluminum determination was as described earlier.

RESULTS

A) TRIFOLIUM REPENS SUBSOIL ROOT DEVELOPMENT AND NUTRIENT COMPOSITION AS INFLUENCED BY SUBSOIL TREATMENT

No subroot development was obtained in the series in which no Ca was applied (Plate I). There was however a remarkable increase in subroot growth when Ca at the rate of 4.5 meq per 100 gm was added to the subsoil. Very little increase in yield was obtained with further increase in Ca level up to 11 meq/100 gm (Table II, Fig. 4). Also, the increase in yield was not much difference between these two Ca levels. Increasing subsoil pH to 5.5 or 6.3 in the Ca treated series did not result in a yield increase in T. repens subroots.

Due to insufficient subroot dry weight for meaningful determination, no chemical analysis was performed for the zero-Ca series. Because of this analysis of variance was carried out for two Ca and three pH levels only (Appendix Table I) instead of three Ca and three pH levels (Appendix Table II).

No significant differences in the P, Al, Ca and Mg concentrations in the subroots were found between the low and the high Ca series. Magnesium concentration in subroots from the pH 5.5 treatment was significantly higher than that for the pH 4.8 treatment (Table II).

B) LOTUS ULIGINOSIS SUBSOIL ROOT DEVELOPMENT AND NUTRIENT COMPOSITION AS INFLUENCED BY SUBSOIL TREATMENT

Dry weight yields of L. uliginosus subroots were increased significantly (1% level) by Ca treatment (Table III, see also Plate II). There was a sevenfold increase in root yield of the two Ca-treated

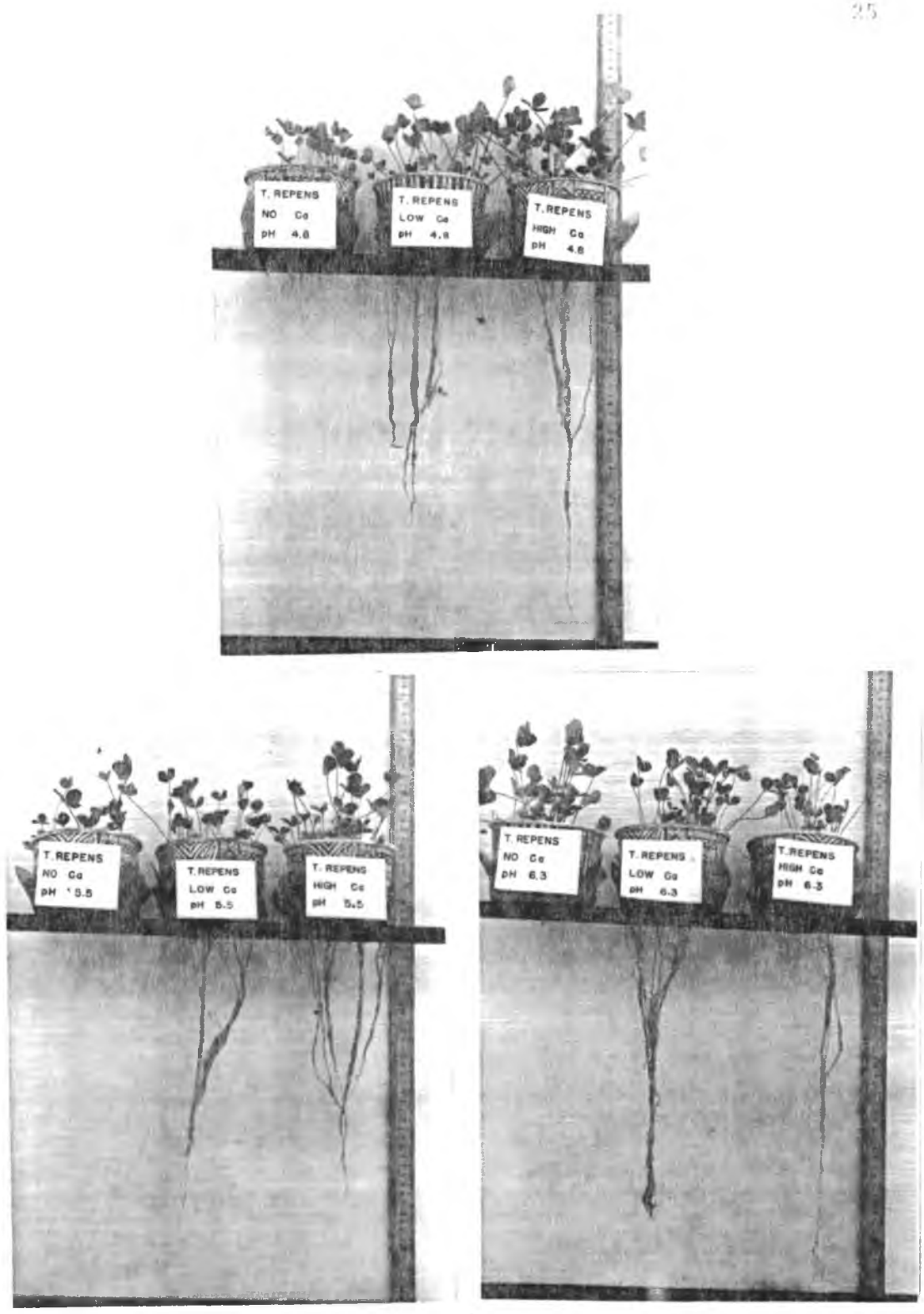


PLATE 1. EFFECTS OF SUBSOIL CA AND PH LEVELS ON SUBSOIL ROOT GROWTH AND DEVELOPMENT OF T. REPENS GROWN FOR 12 WEEKS

TABLE II. THE EFFECTS OF SUBSOIL CALCIUM AND pH ON *T. REPENS* SUBSOIL ROOT GROWTH AND NUTRIENT UPTAKE

Subsoil treatment	Subroot dry wt. mg/pot	Concentration (%)				Uptake (mg/pot)			
		P	Al	Ca	Mg	P	Al	Ca	Mg
0 Ca	10[a]	[b]	-	-	-	-	-	-	-
Low Ca	93	0.14	0.21	0.16	1.19	0.13	0.21	0.15	1.16
High Ca	102	0.15	0.19	0.18	1.20	0.13	0.16	0.19	1.19
pH 4.8	80	0.19	0.20	0.17	0.65	0.12	0.15	0.14	0.48
pH 5.5	110	0.12	0.18	0.15	1.46	0.12	0.16	0.16	1.51
pH 6.3	90	0.13	0.21	0.18	1.48	0.14	0.25	0.21	1.55

[a]Values are mean of 18 samples.

[b]Insufficient subsoil root weight for meaningful analysis.

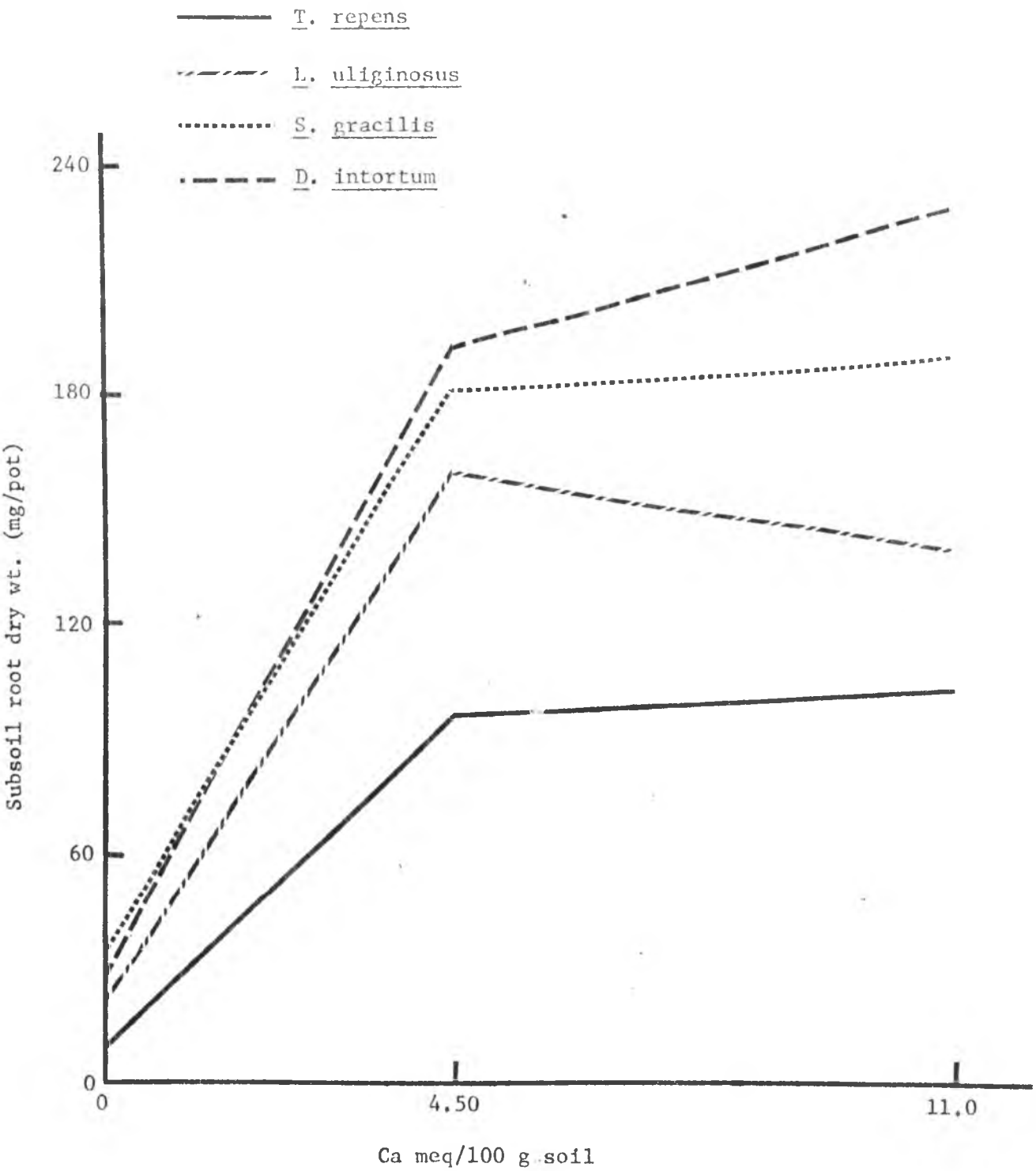


FIG. 4. EFFECTS OF SUBSOIL CA ON THE YIELD OF SUBSOIL ROOTS FOR FOUR LEGUME SPECIES GROWN 12 WEEKS IN PAUWELA SUBSOIL

TABLE III. SUMMARY OF ANALYSIS OF VARIANCE OF GROWTH AND COMPOSITION VARIABLES OF L. ULIGINOSUS SUBSOIL ROOTS

Factor	Subsoil root Dry weight	Concentration in subsoil root				Uptake in subsoil root			
		P	Al	Ca	Mg	P	Al	Ca	Mg
Ca	**	*	*	**		**		**	*
pH			*		**	*			
Ca X pH				*					
Replication			**	*					

*F test significant at 5% level.

**F test significant at 1% level.

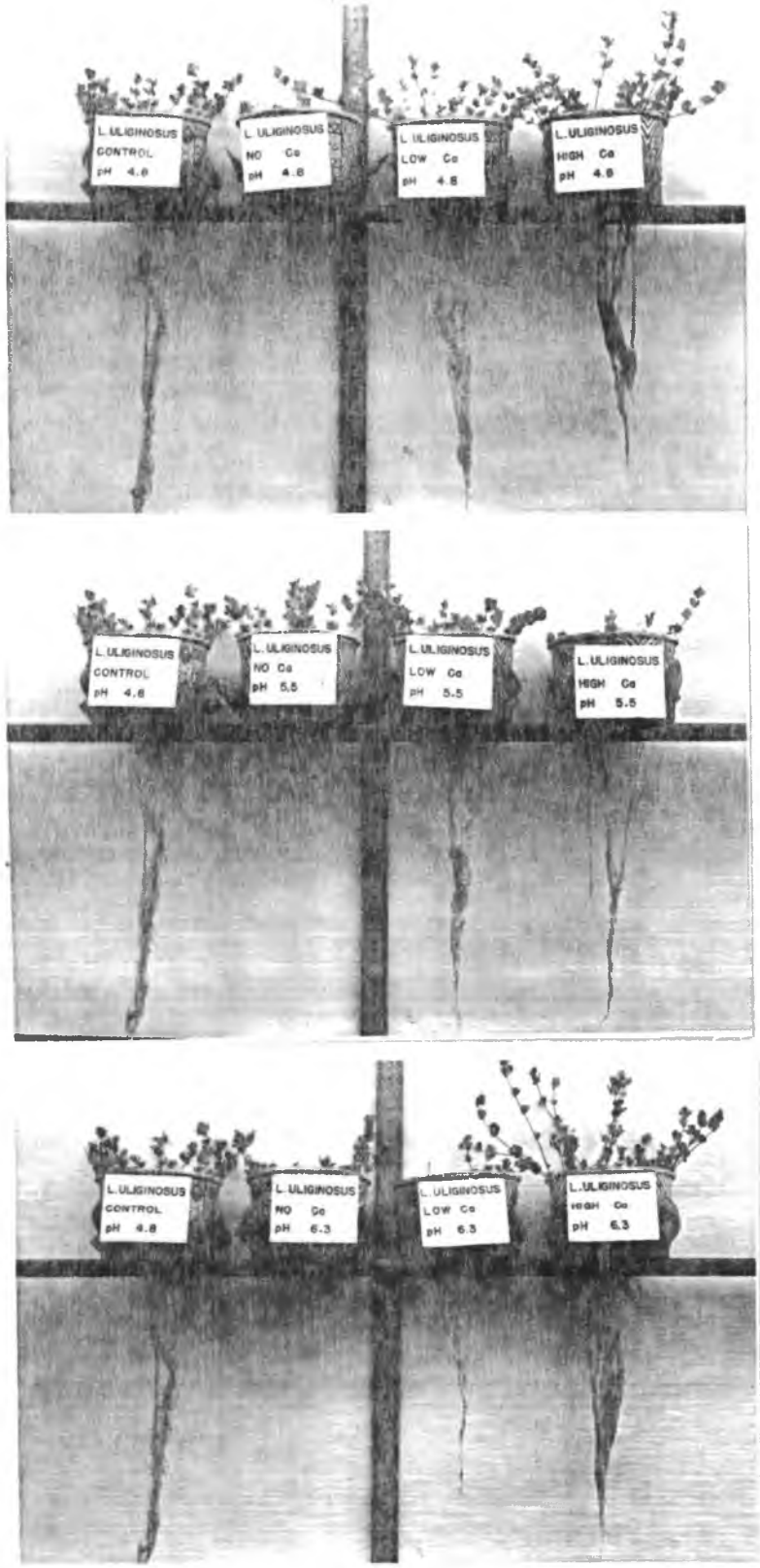


PLATE II. EFFECTS OF SUBSOIL CA AND PH LEVELS ON SUBSOIL ROOT GROWTH AND DEVELOPMENT OF L. ULIGINOSUS GROWN FOR 12 WEEKS

series over the no Ca series. However, there were no significant differences in subroot development between the low and the high Ca levels. Subroot growth was slightly depressed at the high Ca level.

The P concentration in the subroot tissue decreased significantly (1% level) when Ca was added to the subsoil. No significant differences in P concentration were observed between the low and high Ca levels.

The Al concentration in the subroots was significantly affected (5% level) by Ca levels as well as by pH. Calcium at 4.5 meq/100 g generally decreased the Al concentration in the subroots, but there was no further decrease from increasing Ca applications to 11 meq per 100 g (Table IV).

Aluminum concentration in subroots from the pH 6.3 treatment was significantly higher than that in subroots from the other two pH levels, but there was no significant difference in the percentage Al between the pH 4.8 treatment and the pH 5.5 treatment. However, the average aluminum uptake decreased with increased pH as shown in Table IV, possibly because subroot dry weight decreased with pH.

The calcium concentration in subsoil root was significantly influenced by Ca X pH interaction (Table III). The Ca concentration was very low for all the values of pH at the zero Ca level. In the low Ca series, pH 5.5 had a higher Ca concentration than for the other two pH values. But, the Ca concentration was greater for pH 4.8 and pH 6.3 than at pH 5.5 with further Ca addition (Fig. 5).

The Mg concentration in the subroots was not affected by Ca levels. Although increasing applications of Ca to the subsoil would normally be expected to depress Mg uptake due to competition, this was not the case

TABLE IV. THE EFFECTS OF SUBSOIL CALCIUM AND pH ON L. ULIGINOSUS SUBSOIL ROOT GROWTH AND NUTRIENT UPTAKE*

Subsoil treatment	Subsoil root dry wt. mg/pot	Concentration (%)				Uptake (mg/pot)			
		P	Al	Ca	Mg	P	Al	Ca	Mg
0 Ca	22[a]	0.27[a]**	0.44[a]	0.12[a]	0.45[a]	0.04[a]	0.10[a]	0.02[a]	0.09[a]
Low Ca	159[b]	0.15[b]	0.25[b]	0.27[b]	0.49[a]	0.22[b]	0.33[a]	0.47[b]	0.66[b]
High Ca	139[b]	0.15[b]	0.29[b]	0.35[b]	0.47[a]	0.21[b]	0.33[a]	0.53[b]	0.65[b]
pH 4.8	132[a]	0.17[a]	0.29[a]	0.24[a]	0.30[a]	0.21[a]	0.33[a]	0.39[a]	0.40[a]
pH 5.5	127[a]	0.16[a]	0.27[a]	0.25[a]	0.45[b]	0.18[ab]	0.28[a]	0.45[a]	0.59[a]
pH 6.3	59[a]	0.24[a]	0.42[b]	0.25[a]	0.66[c]	0.08[b]	0.16[a]	0.18[a]	0.41[a]

*Values are mean of 27 samples.

**Values which have 'a' letter in common do not differ at the 5% probability level (Duncan's multiple range test).

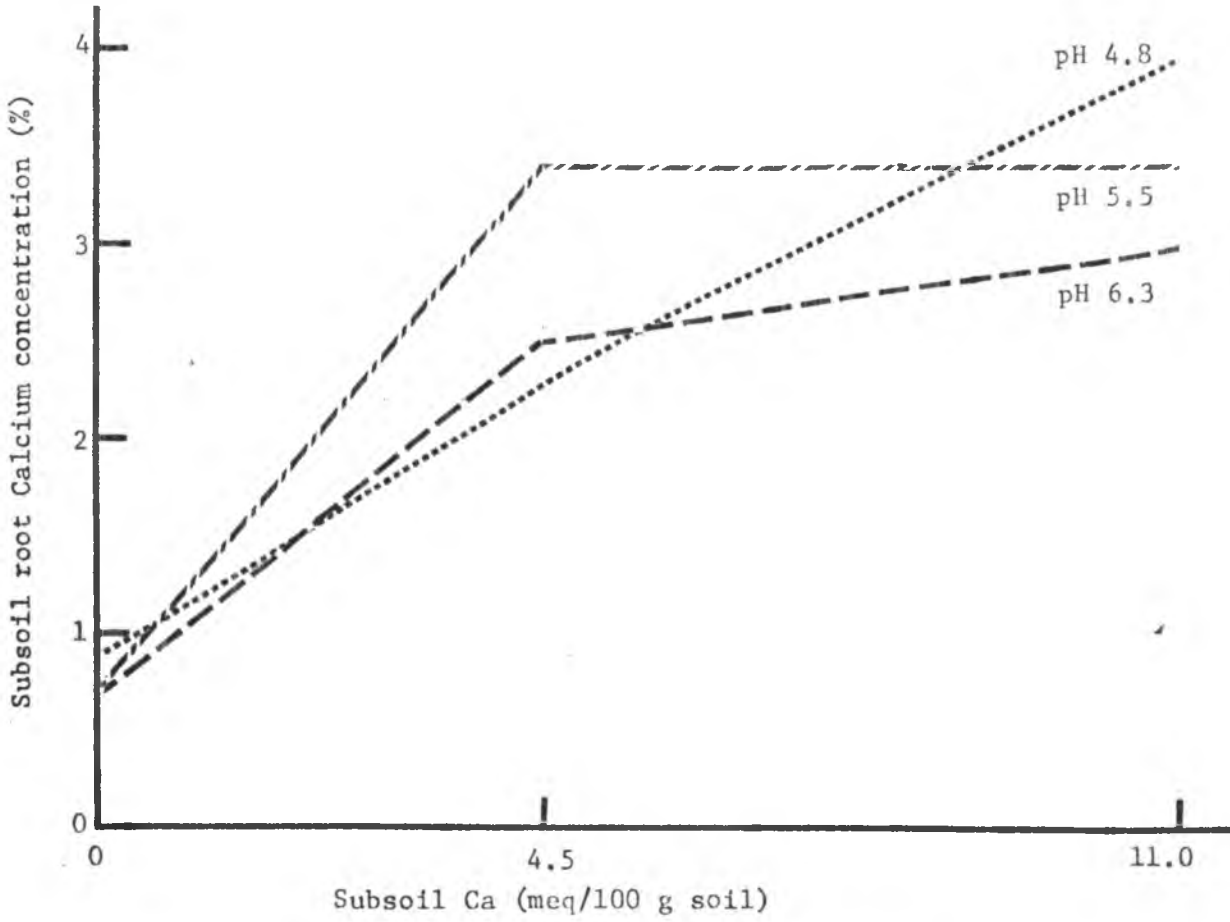


FIG. 5. EFFECT OF SUBSOIL CA X PH INTERACTION ON CA CONCENTRATION IN SUBSOIL ROOT OF L. ULIGINOSUS

as shown in Table IV. The magnesium content in the subroot remained almost constant regardless of Ca level in the subsoil or Ca concentration in the subroot tissues.

The percentage of Mg in the subroots increased significantly (1% level) with increasing pH. The Mg content was significantly different at each pH level.

The relationships between root growth and the various soil and root variables were studied using simple correlation. A summary of correlation coefficient is presented in Table V. The subroot dry weight was highly correlated (1% level) with dry weight of the top roots and of the plant tops as wellas with subroot length, Al content, Ca content, Ca/Mg ratio and Ca/Al ratio, and with subsoil Ca treatment at the 5% level (Fig. 6, 7 & 8). The percentage of Ca in the subroot which was responsible for subroot development (+0.626) was highly correlated (+0.762) with the subsoil Ca treatment. There was a negative correlation between the P, Al and Mg concentration in the subroot with subroot dry weight. The Ca/Mg ratio which was positively correlated with subroot dry weight (+0.579) was also positively correlated with subsoil Ca treatment (+0.660). However a negative correlation was observed between this ratio and pH treatment. The same was true for the Ca/Al ratio. The Mg concentration in the subroot had a significant positive correlation with pH (+0.855).

C) STYLOSANTHUS GRACILIS SUBSOIL ROOT DEVELOPMENT AND NUTRIENT COMPOSITION AS INFLUENCED BY SUBSOIL TREATMENT

The subsoil Ca levels had a significant effect (5% level) upon the dry weight yield of subroot (Table VI). Root development increased

TABLE V. SIMPLE CORRELATION BETWEEN SEVERAL SOIL AND PLANT FACTORS FOR L. ULIGINOSUS GROWN FOR 12 WEEKS

Subroot variables	Subsoil treatment		Subsoil root		Dry weight (mg/pot)	
	Ca	pH	Length	Dry wt.	Toproot	Tops
Dry weight	+0.451*	-0.311	+0.694**	1.000	+0.552**	+0.757**
Root length	+0.756**	-0.069	1.000	+0.694**	+0.003	+0.262
% P	-0.369	+0.217	-0.536**	-0.358	+0.032	-0.091
% Al	-0.281	-0.286	-0.503**	-0.523**	-0.495**	-0.556**
% Ca	+0.762**	+0.014	+0.673**	+0.626**	+0.183	+0.390*
% Mg	+0.025	+0.855**	+0.001	-0.204	-0.257	-0.316
%Ca/%Mg	+0.660**	-0.509**	+0.587**	+0.579**	+0.176	-0.068
%Ca/%Al	+0.511**	-0.091	+0.468*	+0.482*	+0.397*	+0.468*

*For $\geq$ 0.381 $P \leq 0.05$ (d.f. 25)

**For $\geq$ 0.487 $P \leq 0.01$

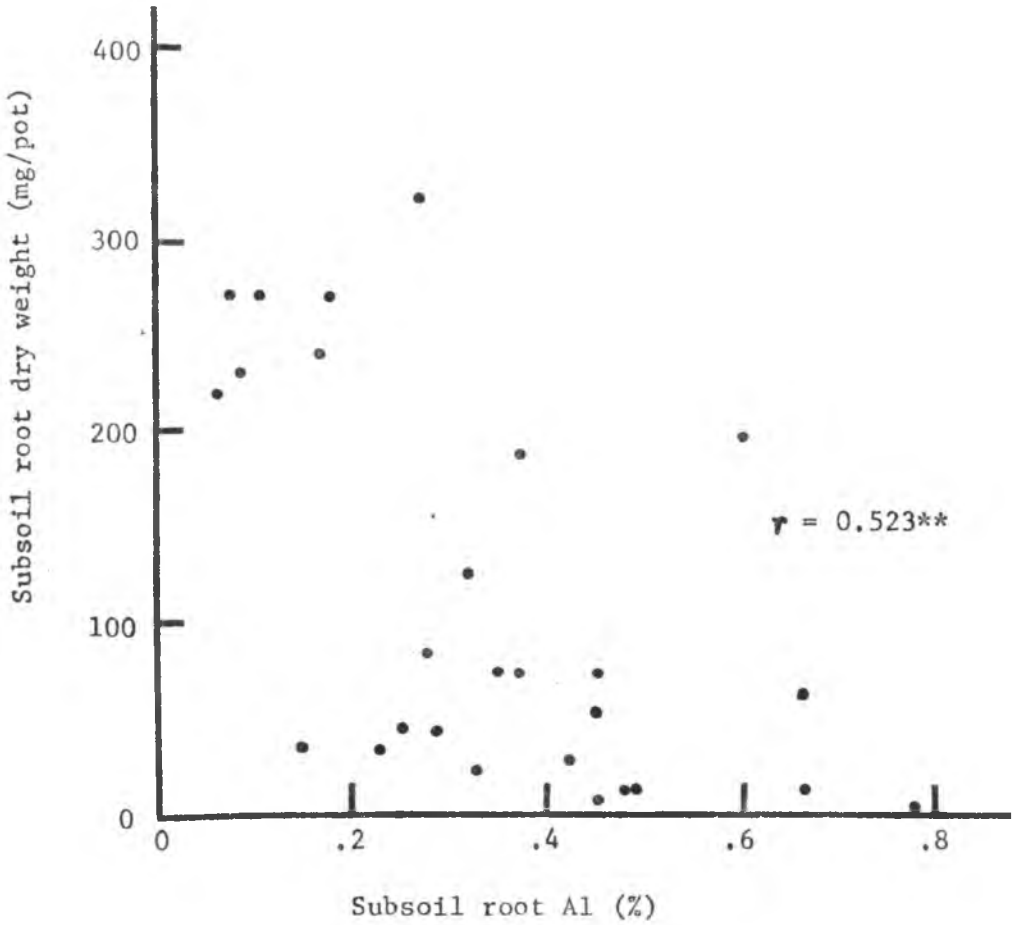


FIG. 6. RELATIONSHIP BETWEEN SUBSOIL ROOT DRY WEIGHT AND SUBSOIL ROOT AL CONCENTRATION OF L. ULIGINOSUS

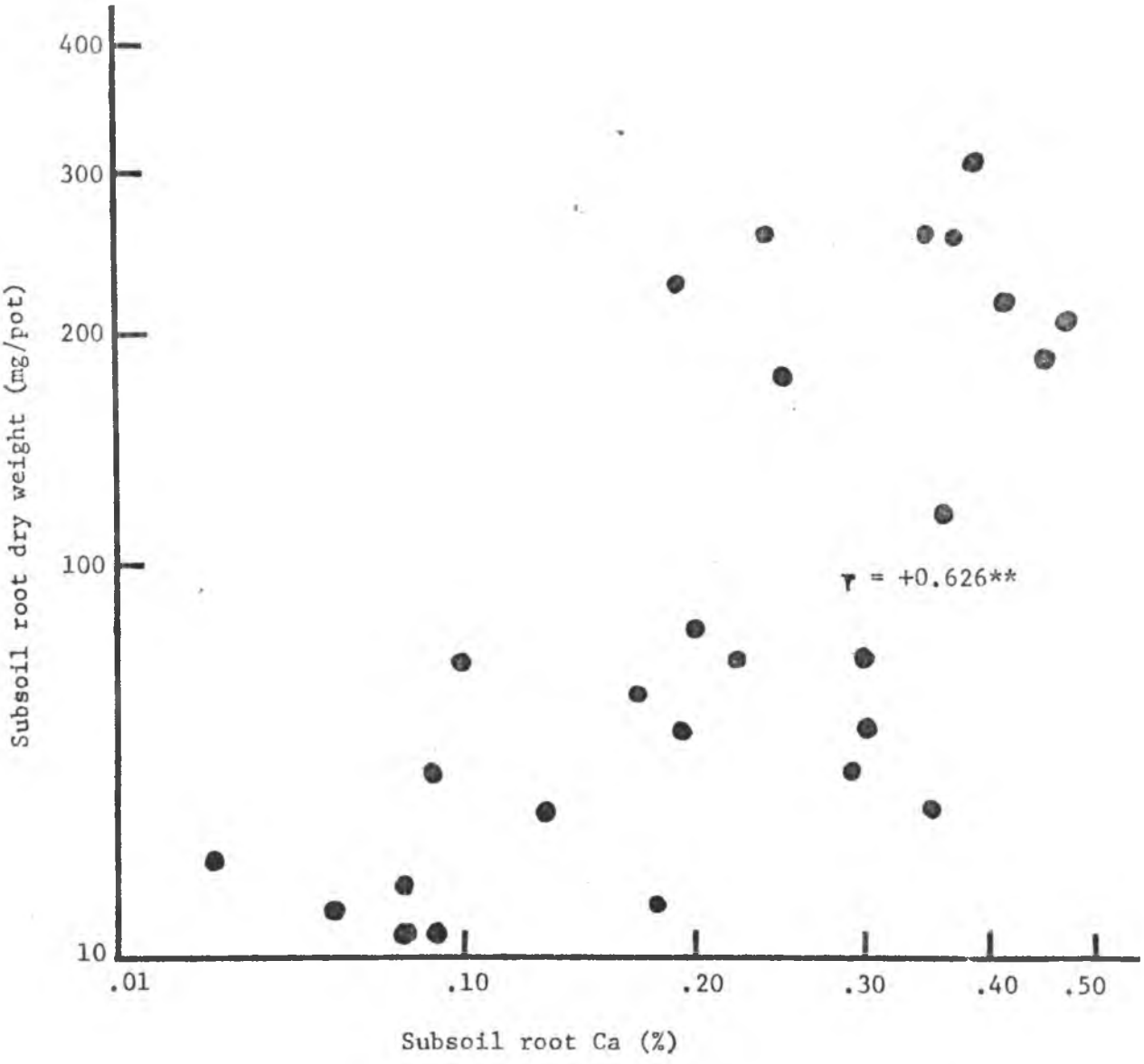


FIG. 7. RELATIONSHIP BETWEEN LOG OF SUBSOIL ROOT DRY WEIGHT AND LOG OF SUBSOIL ROOT CA CONCENTRATION OF L. ULIGINOSUS

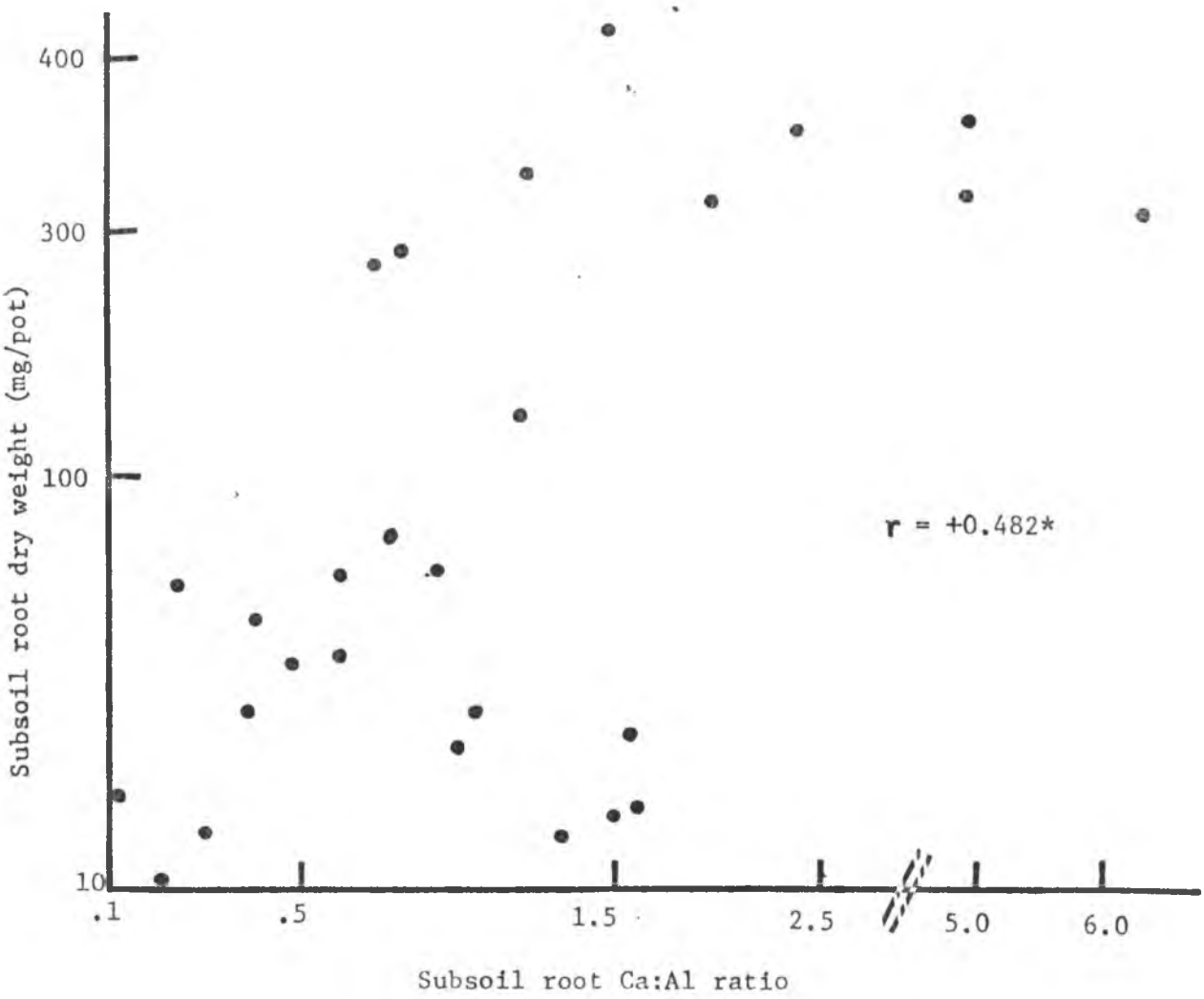


FIG. 8. RELATIONSHIP BETWEEN LOG OF SUBSOIL ROOT DRY WEIGHT AND LOG OF SUBSOIL ROOT CA:AL RATIO OF L. ULIGINOSUS

TABLE VI. SUMMARY OF ANALYSIS OF VARIANCE OF GROWTH AND COMPOSITION VARIABLES OF *S. GRACILIS* SUBSOIL ROOTS

Factor	Subsoil root	Concentration in subsoil root				Uptake in subsoil root			
	Dry weight	P	Al	Ca	Mg	P	Al	Ca	Mg
Ca	*	**		*				*	*
pH		**							
Ca X pH		**							
Replication		*							

*F test significant at 5% level.

**F test significant at 1% level.

five fold when Ca at 4.5 meq per 100 g was added. Further, increasing the Ca level to 11 meq resulted in only a slight increase in subroot growth (Table VII, Fig. 4, Plate III).

The P concentration in the subsoil roots was significantly influenced (1% level) by Ca X pH interaction (Table VI). There was a marked increase in P concentration with increasing pH at the zero Ca series. However no difference in P concentration was found at the low Ca series for all the levels of pH. At the high Ca series, pH 4.8 had a higher P concentration than for the other two levels of pH (Fig. 9).

The concentration Al in the subroot was not significantly affected by the Ca treatment, the pH levels or the Ca X pH interaction. Nevertheless Al concentration in the subroot tended to be somewhat higher in the zero-Ca series (Table VII).

The Ca concentration in the subroots increased as Ca application increased, but only the concentration at the highest Ca application (11 meq Ca/100 g) was significantly higher than that at the zero and low Ca application. The differences in average calcium contents between the low and the high Ca levels seemed to follow quite closely the differences in subroot dry weight yields (Table VII).

The Mg concentration in the subroot was not significantly affected by any of the applied factors. The average magnesium content in the subroot was consistently uniform irrespective of Ca treatments or pH level.

A summary of the correlation coefficients for several roots and soil variables is presented in Table VIII. The subroot dry weight was highly correlated (1% level) with top root dry weight, plant top dry weight and subroot length. It was also correlated at the 5% level with

TABLE VII. THE EFFECTS OF SUBSOIL CALCIUM AND PH ON S. GRACILIS SUBSOIL ROOT GROWTH AND NUTRIENT UPTAKE*

Subsoil treatment	Subsoil root dry weight mg/pot	Concentration %				Uptake (mg/pot)			
		P	Al	Ca	Mg	P	Al	Ca	Mg
0 Ca	36^{a}**	0.34^{a}	0.25^{a}	0.11^{a}	1.1^{a}	0.11^{a}	0.08^{a}	0.03^{a}	0.41^{a}
Low Ca	181^{b}	0.15^{b}	0.17^{a}	0.14^{a}	1.0^{a}	0.26^{a}	0.23^{ab}	0.27^{b}	2.01^{ab}
High Ca	188^{b}	0.15^{b}	0.20^{a}	0.20^{b}	1.2^{a}	0.23^{a}	0.36^{b}	0.33^{b}	2.43^{b}
pH 4.8	100^{a}	0.17^{a}	0.22^{a}	0.15^{a}	1.0^{a}	0.14^{a}	0.16^{a}	0.13^{a}	1.01^{a}
pH 5.5	161^{a}	0.18^{a}	0.22^{a}	0.13^{a}	1.1^{a}	0.21^{a}	0.23^{a}	0.24^{a}	1.89^{a}
pH 6.3	144^{a}	0.28^{b}	0.18^{a}	0.17^{a}	1.2^{a}	0.24^{a}	0.24^{a}	0.27^{a}	1.95^{a}

*Values are mean of 27 samples.

**Values which have 'a' letter in common, do not differ at 5% probability level (Duncan's multiple range test).

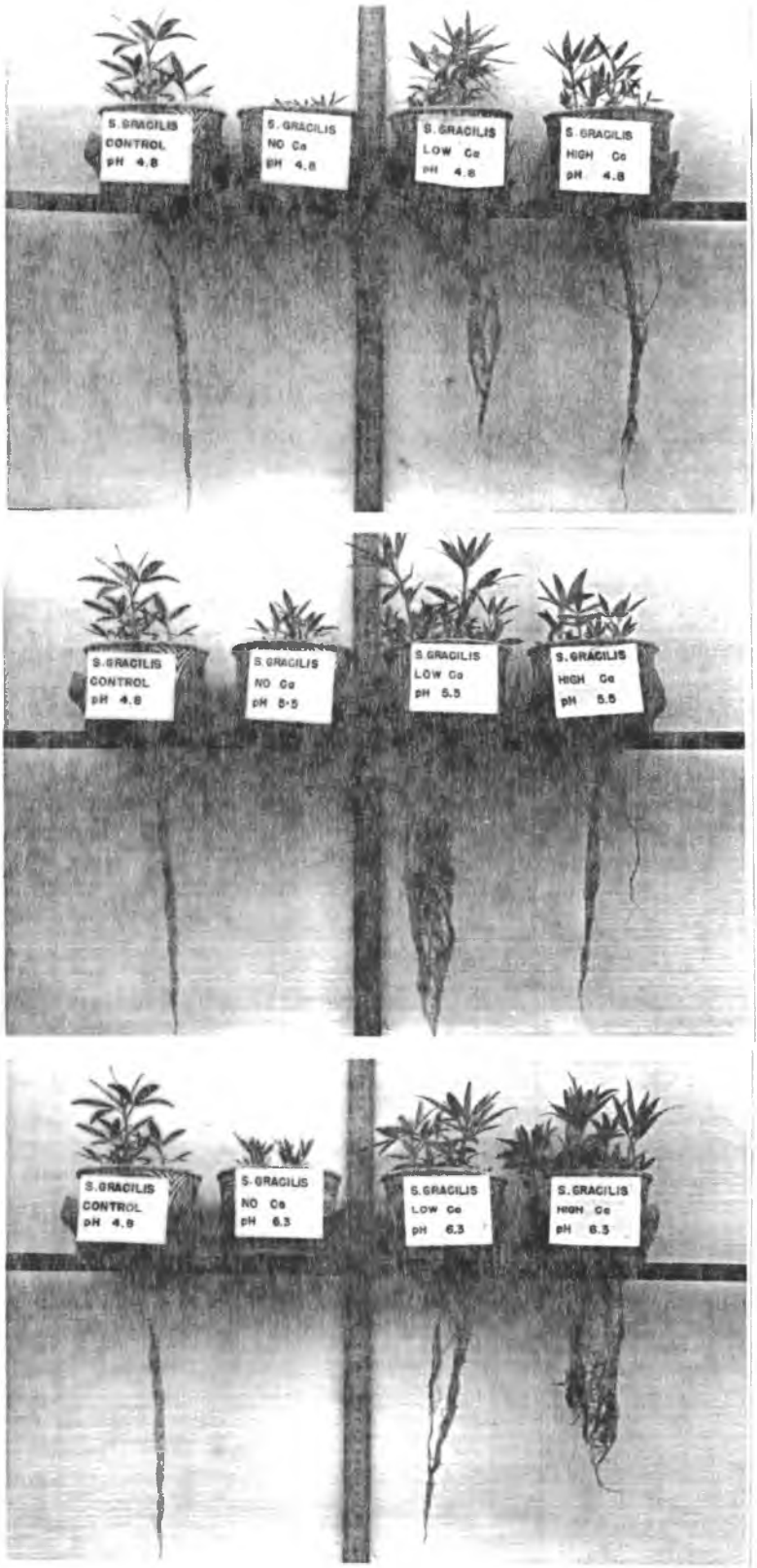


PLATE III. EFFECTS OF SUBSOIL CA AND PH LEVELS ON SUBSOIL ROOT GROWTH AND DEVELOPMENT OF S. GRACILIS GROWN FOR 12 WEEKS

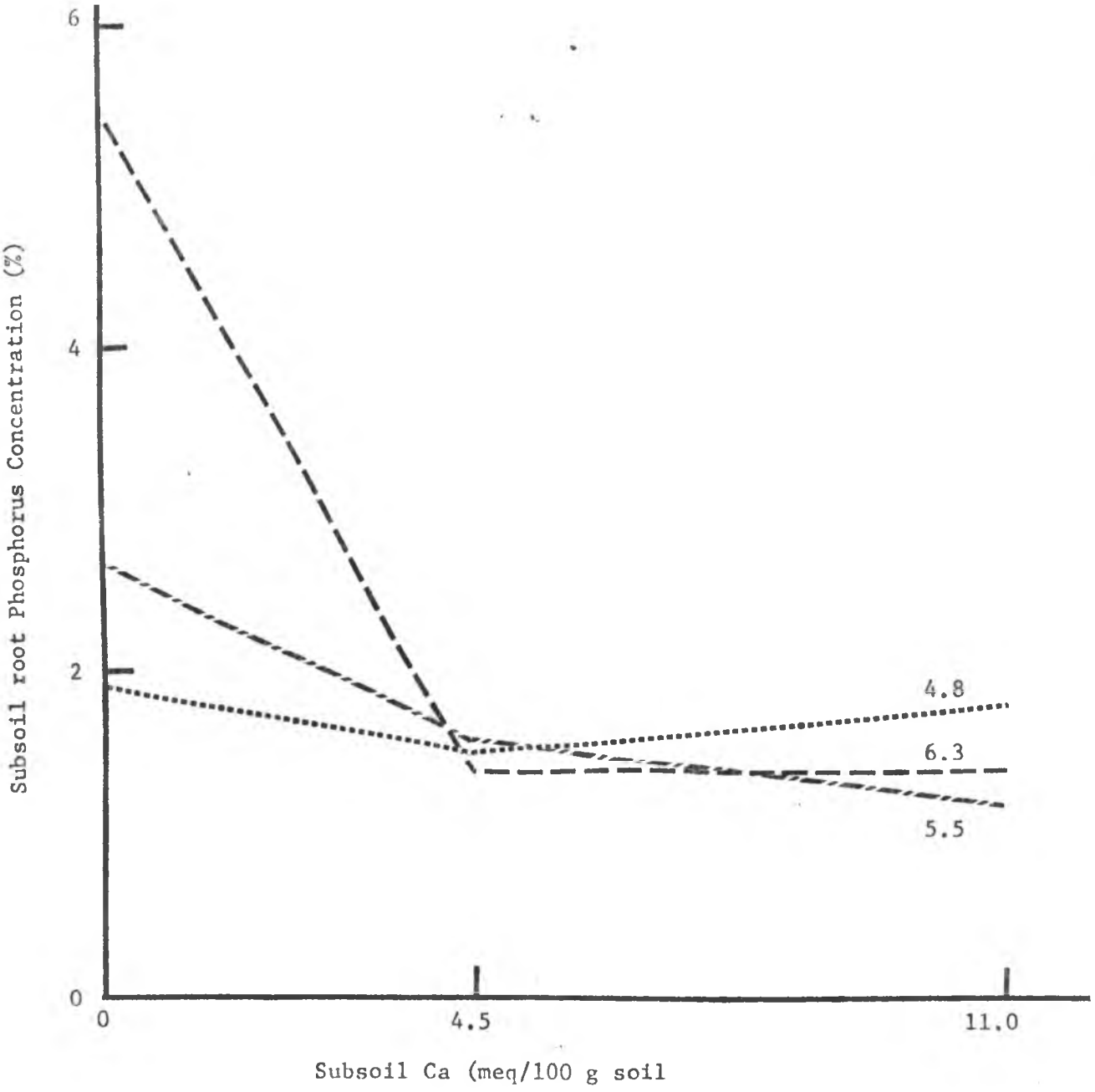


FIG. 9. EFFECTS OF SUBSOIL CA X PH INTERACTION ON P CONCENTRATION IN SUBSOIL ROOT OF S. GRACILIS

TABLE VIII. SIMPLE CORRELATION BETWEEN SEVERAL SOIL AND PLANT FACTORS FOR S. GRACILIS GROWN FOR 12 WEEKS

Subroot variables	Subsoil Treatment		Subsoil root		Dry weight (mg/pot)	
	Ca	pH	Length	Dry wt.	Toproot	Tops
Dry wt.	+0.382*	+0.122	+0.558**	1.000	+0.807**	+0.879**
Root length	+0.540**	-0.057	1.000	+0.558**	+0.221	+0.321
% P	-0.524**	+0.309	-0.662**	-0.437*	-0.210	-0.268
% Al	-0.183	-0.160	-0.492**	-0.410*	-0.278	-0.339
% Ca	+0.543**	+0.133	-0.094	+0.094	-0.037	+0.034
% Mg	+0.231	+0.371	+0.257	+0.403*	+0.466	+0.497**
%Ca/%Mg	+0.298	-0.131	-0.220	-0.175	-0.297	-0.247
%Ca/%Al	+0.487**	+0.262	+0.257	+0.482*	+0.328	+0.377

*For $\geq$ 0.381 $P \leq 0.05$ (d.f. = 25)

**For $\geq$ 0.487 $P \leq 0.01$

subsoil Ca as well as P, Al and Mg concentration in the subroot and also with Ca/Al ratio of the subroot (Fig. 10 and Fig. 11).

The P and Al concentration were negatively correlated with subroot dry weight. There was also a significant negative correlation between P concentration and subsoil Ca. The average Ca content in the subroot was significantly correlated (+0.543) with Ca treatment but not with subroot dry weight. On the other hand, the percentage Mg in the subroot had a significant positive correlation (+0.403) with subroot dry weight but not with Ca treatment (+0.298). The Ca/Al ratio had a significant positive correlation with subroot dry weight (+0.482) as well as subsoil calcium treatment (+0.487). No significant correlation of pH was observed with subroot dry weight nor with any of the other dependent variables (Table VIII).

D) DESMODIUM INTORTUM SUBSOIL ROOT DEVELOPMENT AND NUTRIENT COMPOSITION AS INFLUENCED BY SUBSOIL TREATMENTS

The subroot dry weight was significantly affected (1% level) by the subsoil Ca treatment (Table IX). Root growth in the zero-Ca series was significantly lower (1% level) than in the Ca treated series. There was a further increase in subroot dry weight at the high Ca level but the effect was not significant (see also Plate IV and Fig. 4).

The average phosphorus concentration in the subroot responded significantly (1% level) to the subsoil calcium treatment in the subsoil. The P concentration was significantly higher (1% level) in the zero Ca series over the series in which Ca was added (Table X). However, there was no significant difference in P concentration between the low and high Ca-treated series.

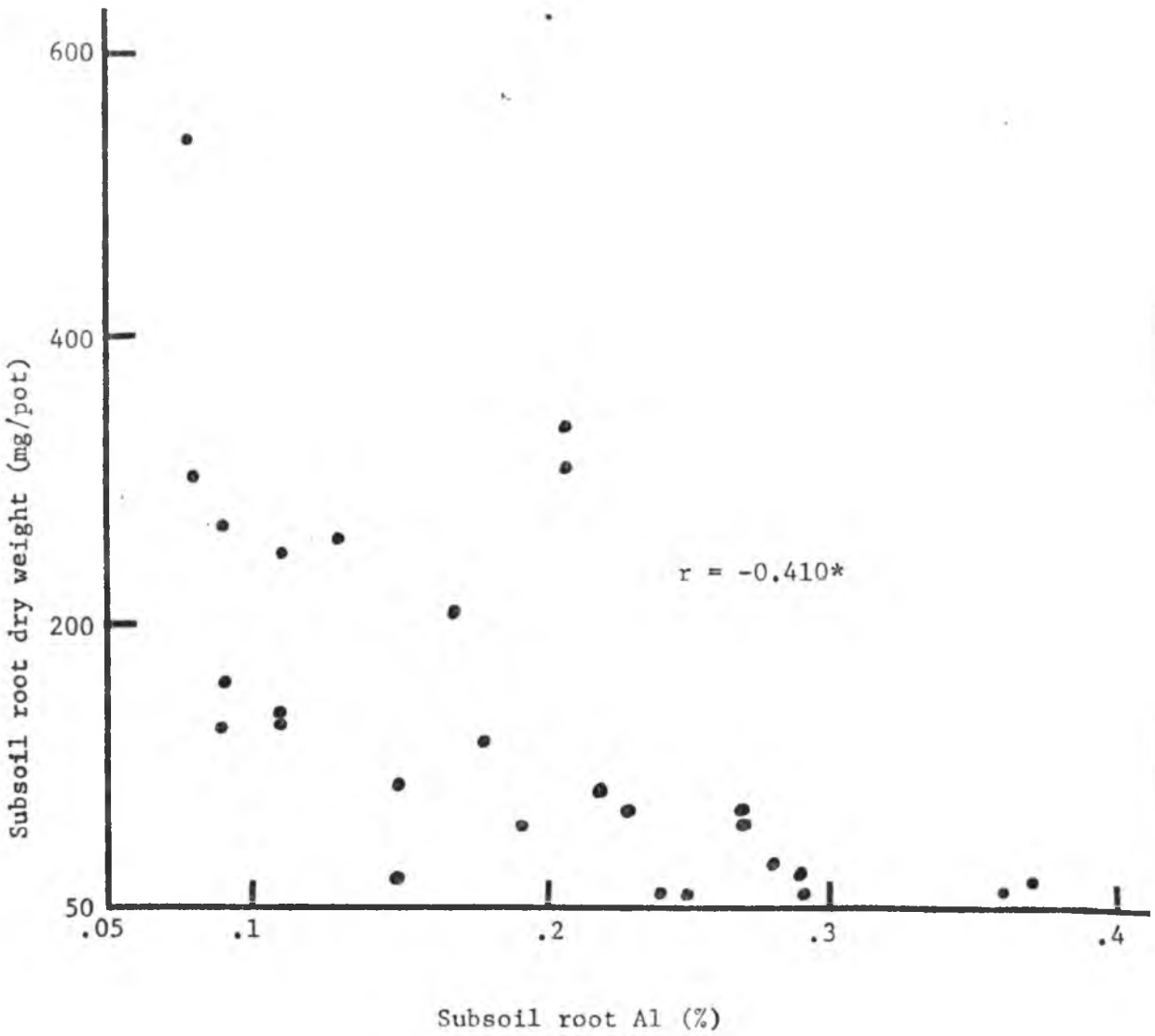


FIG. 10. RELATIONSHIP BETWEEN THE SUBSOIL ROOT DRY WEIGHT AND SUBSOIL ROOT AL CONCENTRATION OF S. GRACILIS

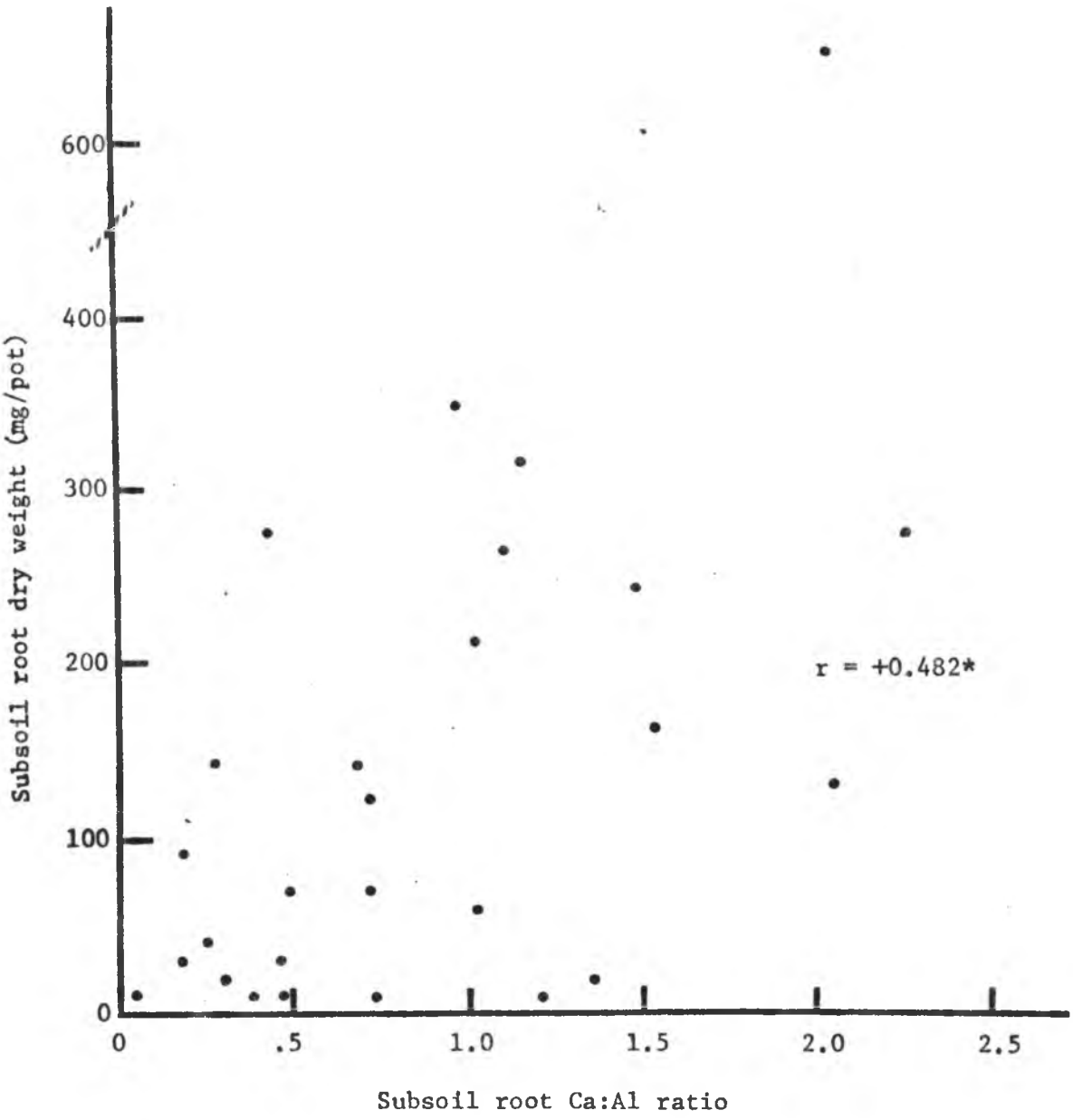


FIG. 11. RELATIONSHIP BETWEEN SUBSOIL ROOT DRY WEIGHT AND SUBSOIL ROOT CA:AL RATIO OF S. GRACILIS

TABLE IX. SUMMARY OF ANALYSIS OF VARIANCE OF GROWTH AND COMPOSITION VARIABLES OF D. INTORTUM SUBSOIL ROOTS

Factor	Subsoil root Dry weight	Concentration in subsoil root				Uptake in subsoil root			
		P	Al	Ca	Mg	P	Al	Ca	Mg
Ca	**	**	**	*	*	**	**	**	**
pH					**				
Ca X pH									
Replication		*							

*F test significant at 5% level.

**F test significant at 1% level.

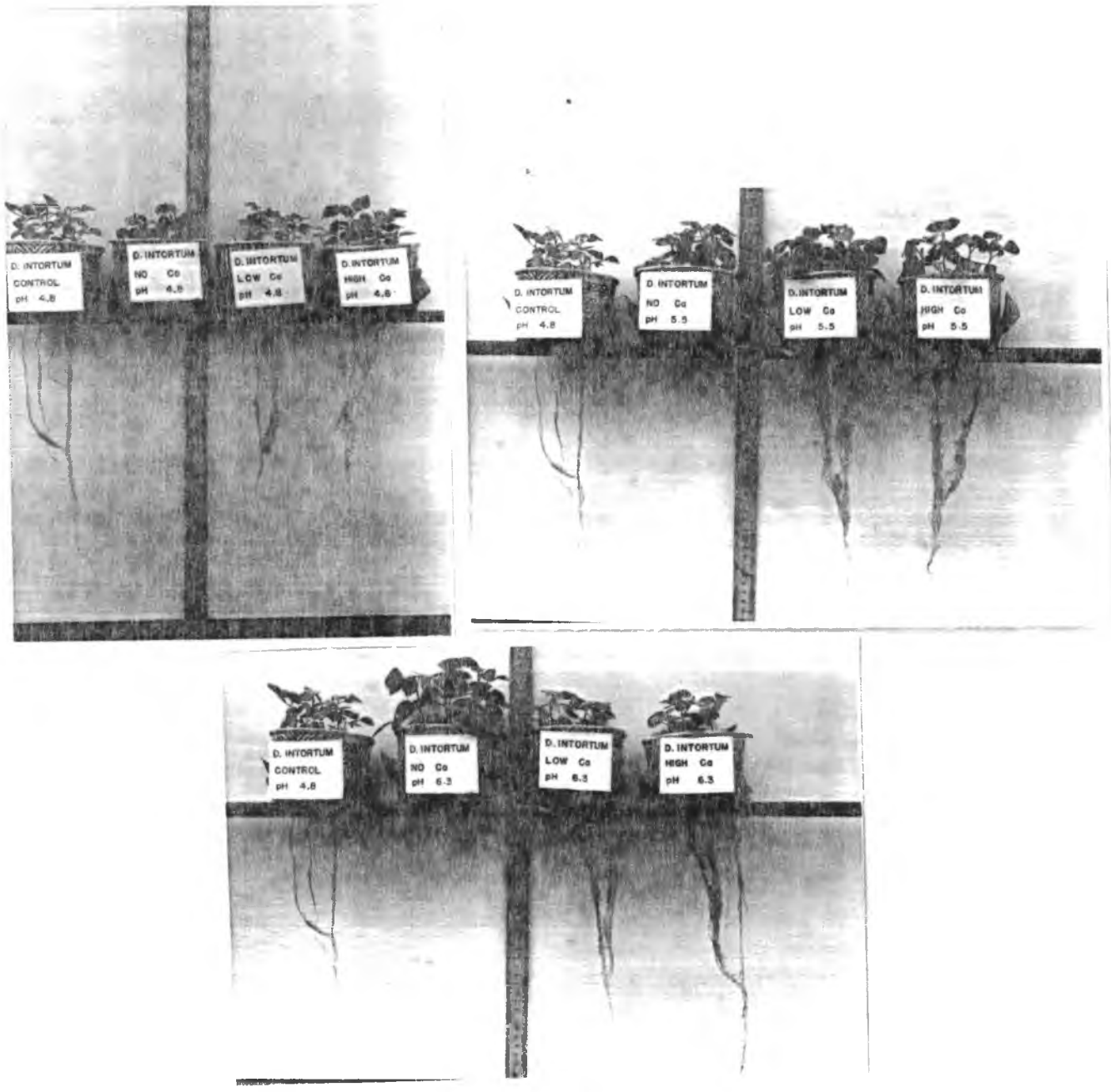


PLATE IV. EFFECTS OF SUBSOIL CA AND PH LEVELS ON SUBSOIL ROOT GROWTH AND DEVELOPMENT OF D. INTORTUM GROWN FOR 12 WEEKS

TABLE X. THE EFFECTS OF SUBSOIL CALCIUM AND PH ON D. INTORTUM SUBSOIL ROOT GROWTH AND NUTRIENT UPTAKE*

Subsoil treatment	Subsoil root dry weight mg/pot	Concentration %				Uptake (mg/pot)			
		P	Al	Ca	Mg	P	Al	Ca	Mg
0 Ca	28[a]**	0.22[a]	0.34[a]	0.07[a]	0.48[a]	0.04[a]	0.10[a]	0.02[a]	0.13[a]
Low Ca	193[b]	0.10[b]	0.19[b]	0.07[a]	0.68[ab]	0.19[b]	0.35[b]	0.14[b]	1.40[b]
High Ca	228[b]	0.11[b]	0.17[b]	0.11[b]	0.74[b]	0.26[b]	0.42[b]	0.25[c]	1.81[b]
pH 4.8	142[a]	0.12[a]	0.25[a]	0.07[a]	0.46[a]	0.17[a]	0.32[a]	0.11[a]	0.81[a]
pH 5.5	173[a]	0.15[a]	0.22[a]	0.08[a]	0.61[b]	0.20[a]	0.32[a]	0.16[a]	1.30[a]
pH 6.3	134[a]	0.16[a]	0.23[a]	0.10[a]	0.84[c]	0.14[a]	0.22[a]	0.13[a]	1.23[a]

*Values are mean of 27 samples.

**Values which have 'a' letter in common do not differ at 5% probability level (Duncan's multiple range test).

A highly significant decrease (1% level) in Al concentration in the subroot was obtained with calcium treatment; Al concentration was significantly higher (1% level) in the series which received no calcium. Addition of Ca to the subsoil decreased the Al concentration absorbed in the subroot, but no significant differences in Al concentration were obtained between the low and the high Ca levels.

The Ca concentration in the subroot responded significantly (5% level) to Calcium treatment. No significant difference in Ca concentration was obtained between the zero-Ca and low-Ca series, but Ca concentration in the high-Ca series was significantly higher than that in either the zero-Ca or low-Ca series.

Calcium level had a significant effect (5% level) on the Mg concentration in the subroot. The Mg content of the subroot in the zero-Ca series was significantly lower (5% level) than that in the Ca-treated series, but there was no significant difference in Mg content of the subroot existed between the low and high Ca series.

The Mg concentration in the subroot was also significantly affected (1% level) by pH and increased significantly (5% level) with each pH increment. Magnesium concentration was highest at the highest pH (6.3) and this was associated with the lowest yields (Table X).

The relationships between Ca and pH treatment, mineral composition of subroots and the dry weight of plant parts are summarized by the correlation coefficient as presented in Table XI. Subroot dry weight was significantly correlated (1% level) with subroot length, dry weight yield of plant tops and percentage Mg in the subroots and with P and Al concentration in the subroots at the 5% level (see also Fig. 12 and Fig. 13). The largest contributing factor in subroot development was not

TABLE XI. SIMPLE CORRELATION BETWEEN SEVERAL SOIL AND PLANT FACTORS FOR D. INTORTUM GROWN FOR 12 WEEKS

Subroot variables	Subsoil Treatment		Subsoil root		Dry weight (mg/pot)	
	Ca	pH	length	Dry wt.	Toproot	Tops
Dry wt.	+0.672**	-0.033	+0.763**	1.000	+0.184	+0.516**
Root length	+0.799**	+0.002	1.000	+0.763**	-0.175	+0.065
% P	-0.483*	+0.182	-0.658**	-0.454*	+0.060	-0.035
% Al	-0.521**	-0.059	-0.619**	-0.469*	-0.131	-0.215
% Ca	+0.413*	+0.265	+0.179	+0.203	-0.049	+0.062
% Mg	+0.395*	+0.591**	+0.417*	+0.544**	+0.170	+0.341
%Ca/%Mg	+0.006	-0.200	-0.156	-0.265	-0.219	-0.262
%Ca/%Al	+0.613**	+0.159	+0.458*	+0.326	-0.065	+0.044

*For $\geq$ 0.381 $P \leq 0.05$ (d.f. 25)

**For $\geq$ 0.487 $P \leq 0.01$

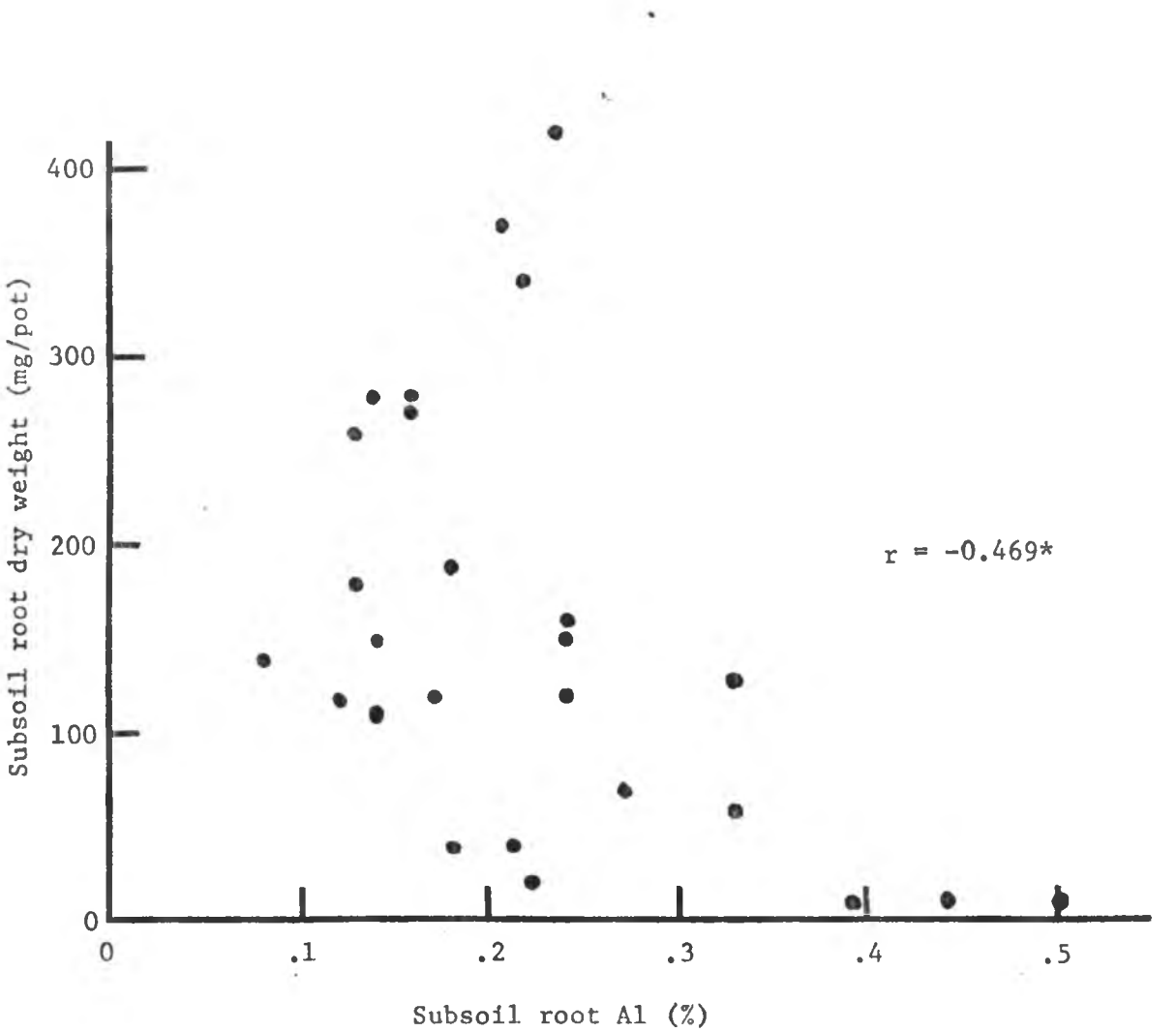


FIG. 12. RELATIONSHIP BETWEEN SUBSOIL ROOT DRY WEIGHT AND SUBSOIL ROOT AL CONCENTRATION OF D. INTORTUM

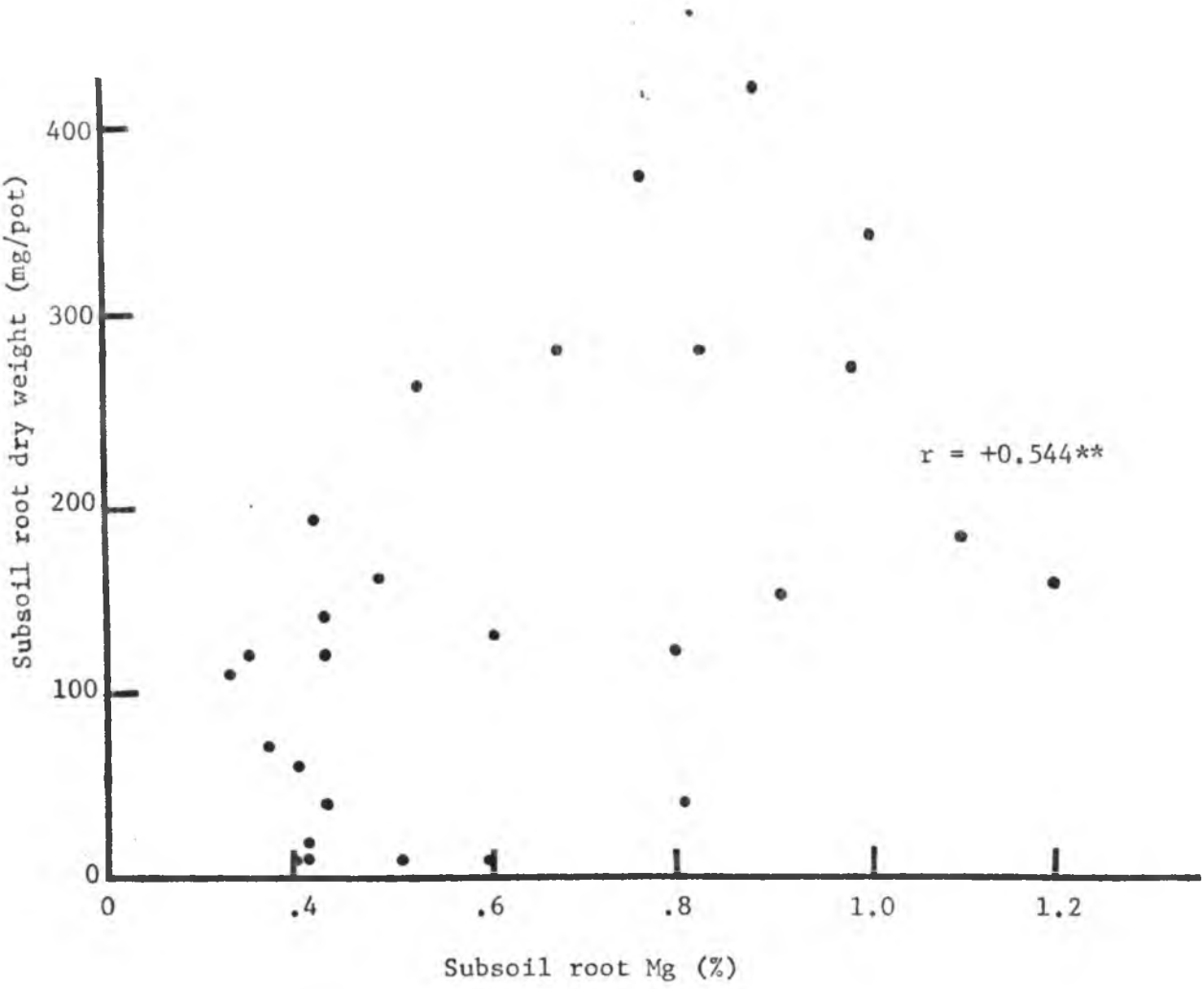


FIG. 13. RELATIONSHIP BETWEEN SUBSOIL ROOT DRY WEIGHT AND SUBSOIL ROOT MG CONCENTRATION OF D. INTORTUM

the amount of Ca content present in the subroot but rather the amount of calcium in the subsoil (+0.672). The Mg concentration in the subroot was positively correlated with subroot dry weight, treatment calcium and treatment pH. The P and Al concentration were negatively correlated with subroot dry weight and also with treatment calcium. Strangely enough the subroot dry weight had a low non-significant positive correlation with the dry weight of roots in the topsoil.

E) UNTREATED CHECKS VERSUS TREATED SUBSOILS (PH 4.8)

The inclusion of the check* pots with the main experiment showed the effects of subsoil treatment** on growth and nutrient uptake of the four legumes. The results are presented in Table XII.

Subroot growth of the four legumes was depressed in the subsoils which received 11 meq Mg/100 g but no calcium (0.0 Ca in Table XII). However, subroot yields increased again when 4.5 meq Ca/100 g was applied to the subsoil (Table XII, Fig. 14). Subroot weight of T. repens and D. intortum increased further increase in subroot weight when 11 meq Ca was applied.

The concentration of phosphorus and aluminum in the subroots (Table XII) did not appear to be greatly influenced by Ca and Mg additions to the subsoil. However, Ca concentration was depressed by the addition of Mg without Ca (except in the case of T. repens), and then increased again as increasing amounts of Ca were supplied. The Ca concentrations in the subroots of L. uliginosus and S. gracilis

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*Refers to original subsoil.

**Contained uniform meq of Mg as $MgSO_4$ to equalize the mineral level (for details see Table I).

TABLE XII. COMPARISON OF EFFECTS OF CHECK AND TREATED SUBSOIL ON SUBSOIL ROOT DRY WEIGHTS AND NUTRIENT COMPOSITION OF FOUR LEGUME SPECIES GROWN 12 WEEKS AT PH 4.8

data - ave. of 3 reps.

Legume species	Treatment	Subsoil root dry weight mg/pot	Concentration in subroots P %	Al %	Ca %	Mg %
T. repens	Check	70	0.13	0.27	0.13	0.22
	0.0 Ca	10	0.12	0.31	0.16	0.67
	4.5 Ca	65	0.16	0.24	0.18	0.65
	11.0 Ca	90	0.22	0.16	0.17	0.64
L. uliginosus	Check	161	0.14	0.23	0.11	0.08
	0.0 Ca	25	0.18	0.28	0.09	0.39
	4.5 Ca	224	0.15	0.21	0.23	0.42
	11.0 Ca	148	0.19	0.37	0.40	0.58
S. gracilis	Check	272	0.18	0.29	0.12	0.10
	0.0 Ca	45	0.19	0.26	0.07	0.32
	4.5 Ca	168	0.15	0.19	0.14	0.26
	11.0 Ca	86	0.18	0.20	0.22	0.32
D. intortum	Check	159	0.13	0.28	0.08	0.37
	0.0 Ca	46	0.16	0.38	0.06	0.99
	4.5 Ca	128	0.10	0.20	0.08	0.75
	11.0 Ca	251	0.12	0.16	0.08	0.99

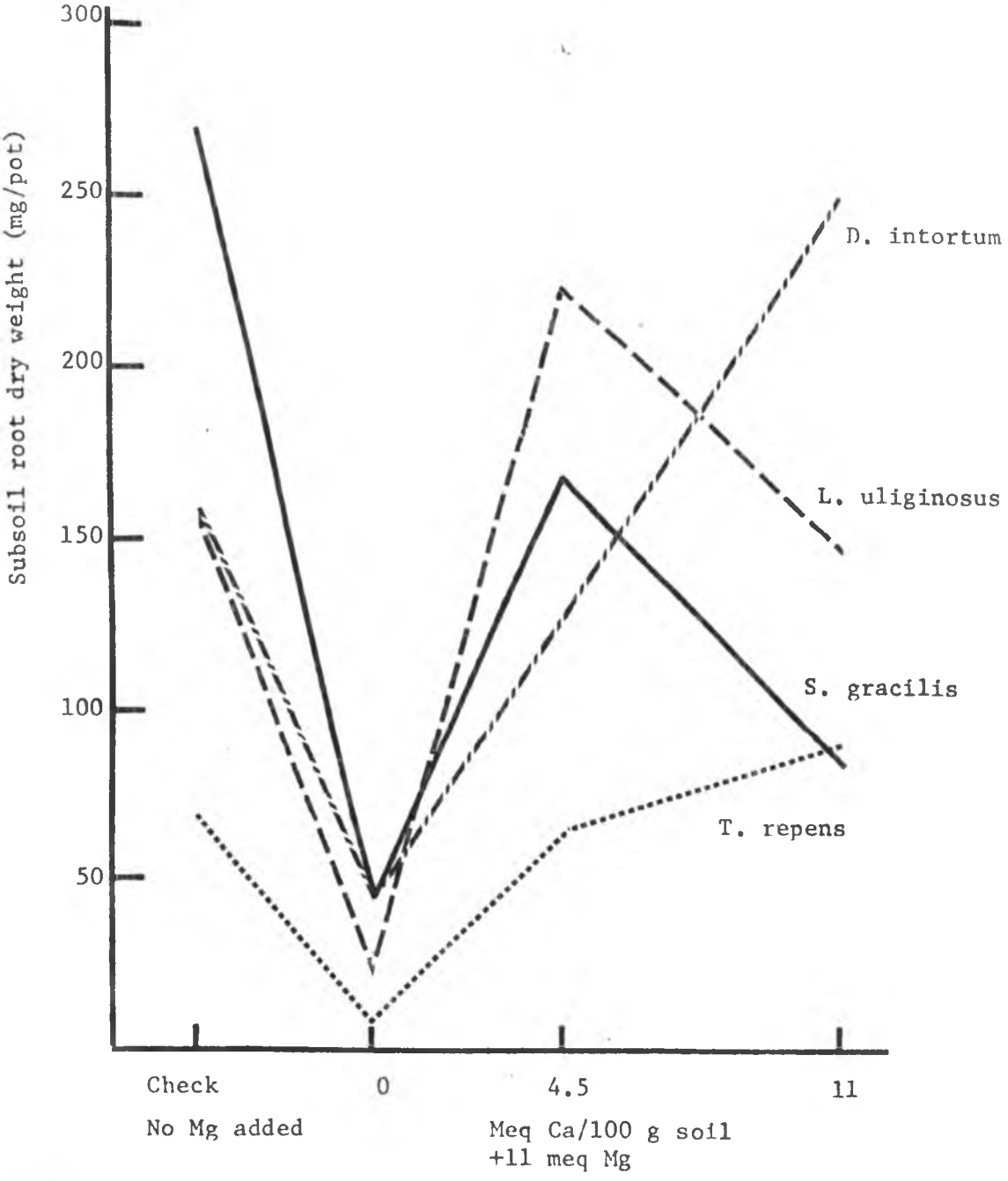


FIG. 14. SUBSOIL ROOT DRY WEIGHT YIELD OF FOUR LEGUMES BETWEEN THE CHECKS AND THE TREATED SUBSOIL AT PH 4.8

were more strongly influenced by subsoil Ca levels than were those of the other two species. Magnesium concentration in the subroots was increased greatly in all four species by the addition of Mg to the subsoil. The addition of Ca up to 11 meq 100 g of subsoil did not appear to greatly affect the levels of Mg in the subroots. The use of Mg in amounts to equalize the mineral level in the subsoil not only depressed subroot growth but also reduced exchangeable Ca, K and Al (Table XIII).

TABLE XIII. SOME CHEMICAL PROPERTIES OF PAUWELA SUBSOIL BEFORE AND AFTER THE EXPERIMENT

Subsoil sampled	pH	C.E.C. meq/100 g	Exchangeable bases			
			Ca	Mg	K	Al
			meq/100 g			
Check:						
start	4.8	23.03	0.35	0.35	0.17	2.50
end	4.7	22.16	0.43	0.28	0.14	1.20
Plus Mg*						
end	4.4	20.93	0.13	6.87	0.12	1.04

*11 meq Mg as $MgSO_4$ incorporated into the subsoil to give constant Mg levels in all treatments except the check.

DISCUSSION

GENERAL EFFECTS DUE TO SUBSOIL MG, CA AND PH LEVELS

In the present study, the addition of Mg at the rate of 11 meq/100 g soil (to equalize Mg levels in all treatments, Table I) induced a considerable change in subsoil conditions (Table XIII). The exchangeable Mg in the subsoil increased by as much as 20 times, from 0.35 meq to 6.87 meq per 100 g soil. This drastic increase in exchangeable Mg reduced exchangeable Ca to an extremely low level in the subsoil. Further, a substantial reduction in exchangeable K and Al was also observed. Since the amount of Mg added was fixed, the effects of Mg addition were assumed to be constant in all of the Ca and pH treatments.

The poor subroot growth in soils receiving Mg compared to the check (Table XII) reflected the detrimental effect of high Mg on subroot development. This was due to the fact that the pre-existing deficiency of Ca (0.35 meq/100 g) was further aggravated by addition of the large amount of Mg (Table XIII). The lack of subroot development in Mg-treated subsoil was thus apparently due to severe nutritional imbalance; predominantly low Ca, rather than to Mg toxicity.

The improved subroot growth in soils receiving Ca as well as Mg demonstrated clearly the beneficial effect of Ca (Table XII, Fig. 14). The same explanation appears valid for the further improvement in subroot growth at the high Ca level.

Although the data in Table XII were based on the results obtained at one pH level, a similar effect would undoubtedly be observed for the

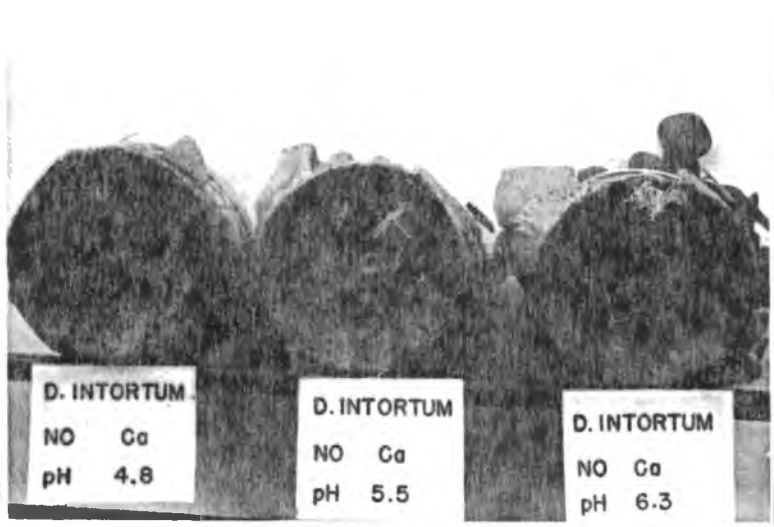
other pH levels since the Mg level was constant in all the treatments.

Adjustment of soil pH in the field is commonly carried out by the application of calcium hydroxide or carbonate which apparently have no deleterious effect. Magnesium carbonate was used in this study specifically for the purpose of adjusting pH while avoiding the use of calcium. The results on the calcium treatments indicate that magnesium can be substituted for calcium compounds for pH adjustment in the field provided other nutrients such as calcium are also added.

EFFECT OF CA LEVELS ON SUBSOIL ROOT GROWTH

The relationship between Ca supply and subroot growth was clearly evident in the results presented in Fig. 4 and Plates V and VI. Subroot development into the subsoil material was inhibited almost completely in the absence of Ca (Tables II, IV, VII and X). Where subroot growth was seriously limited, the roots appeared brown and coral-like in appearance with laterals appearing as peg-like projections (Plate VII). These symptoms are commonly associated with Al toxicity. Many of the Al toxicity symptoms on roots are quite similar to Ca deficiency symptoms (Hallsworth and Greenwood, 1957 and Rorison, 1958). Further, microscopic observations revealed that the growing tip had been severely damaged (Plate VII). In most cases, root tip die back of the longer laterals was observed. Presley and Leonard (1948) in cotton germination tests, also found severe injury to seedling radicles when Ca was deleted from the germination medium. In a severely Ca-deficient soil such as the zero-Ca series, Ca apparently was not absorbed by the subroots at a rate sufficient to maintain normal cell division and lateral root elongation.

PLATE V. SUBROOT DEVELOPMENT OF T. REPENS GROWN IN PAUWELA SUBSOIL AT ZERO-CA AND THREE PH VALUES FOR 12 WEEKS



(a)

(b)

PLATE VI. SUBROOT DEVELOPMENT AND DISTRIBUTION OF D. INTORTUM GROWN IN PAUWELA SUBSOIL TREATED WITH ZERO CA AND THREE PH LEVELS (a) AND 4.5 MEQ CA PER 100 G SOIL AT PH 5.5 (b)

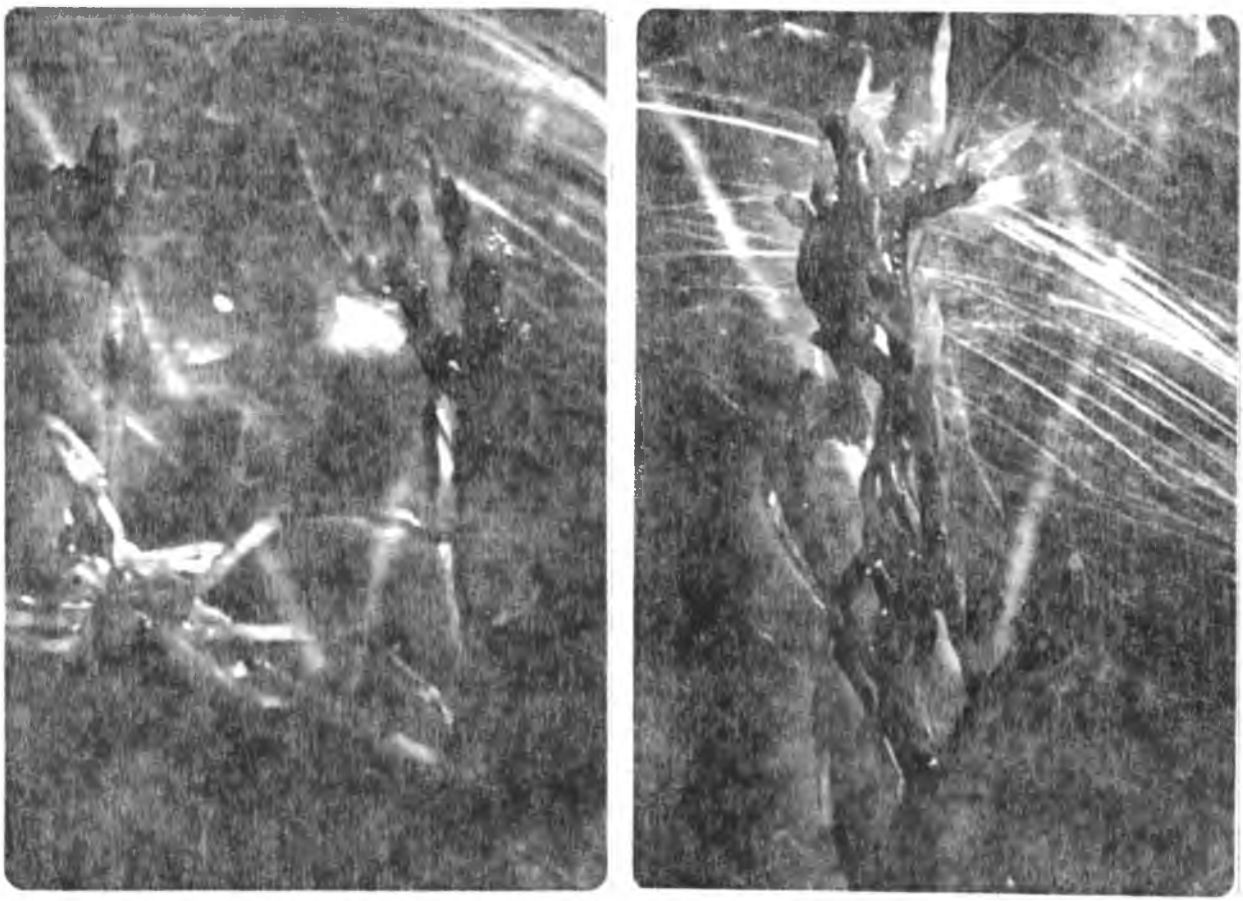


PLATE VII. TYPICAL SUBSOIL ROOT RESPONSE OF D. INTORTUM TO PAUWELA SUBSOIL IN ZERO-CA LEVEL AT PH 4.4

In the present study, the addition of $CaCO_3$ or $CaSO_4$ (4.5 meq Ca per 100 g soil) to the acid Pauwela subsoil with 11 meq Mg per 100 g increased subroot development more than six-fold in all the four species (Tables II, IV, VII, and X). It was observed (Fig. 4) that subroot growth of L. uliginosus was depressed at the high Ca level (11 meq Ca per 100 g soil). No explanation can be offered for this. Nevertheless the roots found in these two seires were finely divided and well-branched compared to the thick, stubby root systems in the zero-Ca series (Plate VII). Unlike the checks, the subroots in the Ca-treated series also were not easily broken during the washing operation. Therefore it may be presumed that calcium is associated with the membraneous structure of the cells as reported by Burling and Jackson (1965) and Handley, et al. (1965).

The $CaCO_3$ additions had a two-fold effect on subroot growth, viz:

i) reduction of Al toxicity through the precipitation of Al as calcium aluminate.

ii) increasing the Ca supply to the roots

Subroot development is a function of subsoil environment (as shown in Appendix Table V). Thus regardless of the Ca level in the surface soil, no root growth was possible in the subsoil layers unless Ca was added. This agrees with the work of Albrecht and Davis (1929) who reported that the beneficial effect of lime upon root growth was localized in the zone in which lime was applied. Estrada and Cummings (1968) also reported that lime application to the A_2 horizon resulted in increases in root (Maize) growth but further increase was not

evident from lime application to the Ap horizon.

There was no striking increase in the quantity of roots found in the top soil layers when root growth in the subsoil layer was at a maximum (Appendix Tables V and VI). Root growth of T. repens, L. uliginosus and D. intortum was maximum in the top soil where no Ca was added to the subsoil. The weight of topsoil roots then decreased with addition of Ca to the subsoil. The decrease in roots in the top soil was compensated for by increased growth of roots in the subsoil when Ca was added. As a result, the total root growth in the two soil layers was not greatly different with respect to treatment within species particularly in the case of T. repens. The total root growth was apparently more closely related to the amount of aerial tissue than to soil conditions affecting the growth of subroots, per se.

EFFECT OF PH LEVELS ON SUBSOIL ROOT DEVELOPMENT

The levels of subsoil pH tested did not significantly affect subroot growth in any of the four legume species (Table II, Plate I; Table III, Plate II; Table VI, Plate III; Table IX, Plate IV). Similarly, this was indicated by the fact that there were no significant correlations between subroot dry weight and pH. The effect of Ca was considerably greater than that of pH as indicated by the high significant positive correlation between subroot yield and applied Ca (Tables V, VIII and XI). However, pH appeared to have its maximum effect on the exchangeable Al in the subsoil (Appendix Table XI).

The primary effect of pH treatment was on the nutrient concentration in the subroot tissue (Tables III, VI and IX). The increase in soil pH associated with the application of Ca increased the

availability of P and this presumably influenced the P concentration in L. uliginosus, D. intortum and S. gracilis subroots. In addition treatment pH was positively correlated (1% level) with subroot dry weight in D. intortum and negatively correlated (1% level) with subroot dry weight of L. uliginosus.

THE RELATIONSHIPS BETWEEN SUBSOIL ROOT DEVELOPMENT AND NUTRIENT CONCENTRATIONS IN THE SUBSOIL ROOT

The Mg concentration was high in subroots from all treated subsoils (especially those of T. repens and S. gracilis) but was relatively constant within species regardless of the subsoil Ca level (Tables II, IV and VII). Therefore the Mg concentration of subroots growing in soil containing high Mg is largely determined by plant species, and the level of Mg accumulation in the subroots indicates the tolerance for the species. On this basis, S. gracilis may be classed as a high Mg-tolerant legume since its subroots contained up to 1-1% Mg (Table VII). The same holds true for T. repens on the basis of subroot Mg content at the low and high Ca levels (Table II). By contrast L. uliginosus would be classed as having low tolerance to Mg (0.46% Mg in the subroots, Table IV). Unlike the other three species, the Mg concentration in D. intortum subroots varied with Ca level (Table X) and was in a range that might cause this species to be classified as having medium tolerance to Mg.

The phosphorus content in the subroot material was uniform at 0.15 per cent irrespective of the Ca level in the treatment except for D. intortum subroots which had 0.11% P. It is apparent:

i) that the 500 ppm P applied to the topsoil provided adequate P for subroot development,

ii) that further root development was independent of phosphorus in the subsoil when the phosphorus in the root was maintained above a critical level.

Rios and Pearson (1964) have shown, using a vertical split-root technique, that cotton root development was not inhibited by the absence of P in the rooting medium.

The effects of P, Al and Ca concentration in the subroot on subroot development are interrelated. The significant increase in P content of the stunted subroots recovered from the zero-Ca series (Tables IV, VII and X) indicates that some P was being immobilized in the roots or precipitated on the roots, presumably by aluminum as suggested by Wright (1943), Randall and Vose (1963) and MacLeod and Jackson (1965). This is further demonstrated by the inverse correlation of subroot dry weight and P in L. uliginosus ($r = -0.358$), S. gracilis ($r = -0.437$), and D. intortum ($r = -0.454$).

The inverse relationship could be caused by dilution effects since a high P concentration exists with a relatively small amount of subroot dry weight especially in the zero-Ca level.

As indicated in the review of literature, rather small concentrations of Al-ions in nutrient solution inhibit root growth. In the present study, the existence in both the subroots and the subsoil of Al concentrations which are considered toxic in solution culture experiments is evidence that aluminum toxicity may be occurring in this soil. This is particularly true with the zero-Ca series as

presented in Table XIV showing that subroot growth was inversely proportional to exchangeable Al or % Al saturation in the soil (Ragland and Coleman, 1959).

An exchangeable Al value of 33 ppm or 1.2% Al saturation was found to be toxic in all four legume species. However they differ in their degree of tolerance. Root development into the subsoil was completely inhibited with T. repens whereas small amounts of roots were found with L. uliginosus, S. gracilis and D. intortum.

It was observed that Al content in the subroot was relatively high when high amounts of exchangeable Al was present in the soil. The high percentage of Al was probably due to uptake and precipitation within the roots, to surface adsorption, and also possibly precipitation on the outer surface of the root. Although precautions were taken to remove the surface adsorbed Al during washing, it is not known how much of the total aluminum determined was due to surface adsorption.

The addition of 4.5 meq Ca per 100 g resulted in lowering of both the exchangeable Al in the soil (Table XIV) and the Al concentration in the roots (Tables II, IV, VII and X). The reduction was accompanied by a large increase in subroot growth in all four species (Fig. 4). Further increase in Ca level (to 11 meq/100 g) resulted in further decrease in exchangeable Al in the soil. However the Al concentration in the roots did not differ markedly between the high and low Ca levels. Also the small additional decrease in exchangeable Al with the high Ca generally did not result in further increase in subroot growth, although some increase in root growth was observed in T. repens and D. intortum. The decrease in exchangeable Al due to high Ca addition did not necessarily give a lower percentage of Al in the subroot as indicated

TABLE XIV. EFFECT OF SUBSOIL CA LEVELS ON EXCHANGEABLE SOIL AND SUBROOT AL AND CA CONCENTRATION AND THEIR RELATIVE INFLUENCE ON SUBSOIL ROOT DRY WEIGHT YIELD OF FOUR LEGUME SPECIES GROWN 12 WEEKS IN PAUWELA SUBSOIL*

Species and treatments**	Subsoil root dry weight mg/pot	Exchangeable Al in subsoil (ppm)	Concentration in subroots Al %	Ca %
I. repens				
0.0 Ca	0	33	-	-
4.5 Ca	93	27	0.21	0.16
11.0 Ca	102	17	0.19	0.18
L. uliginosus				
0.0 Ca	22	32	0.44	0.12
4.5 Ca	159	29	0.25	0.27
11.0 Ca	139	16	0.29	0.35
S. gracilis				
0.0 Ca	36	33	0.25	0.11
4.5 Ca	181	25	0.17	0.14
11.0 Ca	188	15	0.20	0.20
D. intortum				
0.0 Ca	28	31	0.34	0.07
4.5 Ca	193	22	0.19	0.07
11.0 Ca	288	15	0.17	0.11

*Ave. over 3 reps. and 3 pH levels.
**Ca levels indicate meq Ca/100 g added to subsoil. All treatments have 11 meq Mg/100 g.

by the data for L. uliginosus and S. gracilis (Table XIV). The results show that Al concentration in the subroot was negatively correlated with subroot growth in the legume species, viz. L. uliginosus (Table V, Fig. 6); S. gracilis (Table VIII, Fig. 10) and D. intortum (Table XI, Fig. 12). T. repens is not included because of the absence of roots. Some of the detrimental effects of Al on root growth in the low pH treatment have been attributed to inhibition of Ca uptake (Johnson and Jackson, 1964). The results of this experiment indicate that both factors might be operating together against the legumes susceptible to aluminum toxicity.

"Aluminum toxicity," as related by Mum (1965), "can be distinguished because it characteristically inhibits root growth, leads to high al inum concentrations in the plants and is readily remedied by liming." This description fits the data in Table XIV quite well.

The data in Tables II, IV, VII and X indicate that at the zero-Ca level a low Ca content in the subroot was associated with high concentration of Al and P in the subroot which seriously inhibited the subroot development. Under these conditions exchangeable Ca in the soil was only 0.13 meq/100 g (or 0.62% Ca saturation).

Since Ca concentration in the roots was positively correlated with Ca level in the soil, it appears that inhibition of Ca uptake due to high soil Al can be alleviated by the addition of Ca (Table XIV). Subroot Ca level was negatively correlated with Al concentration in the root in all the legume species, but the correlation was significant only in the case of D. intortum. At pH 4.8 in the zero Ca treatment, the poor subroot growth of all species may be due to either or both

factors of Al toxicity and Ca deficiency. It is well known (Rorison, 1958, Fig. 15, and Appendix Table XI) that Al precipitates as the pH is elevated to 7.0. Therefore the depressed root growth at all pH levels in the zero Ca treatment is definitely indicative of Ca deficiency.

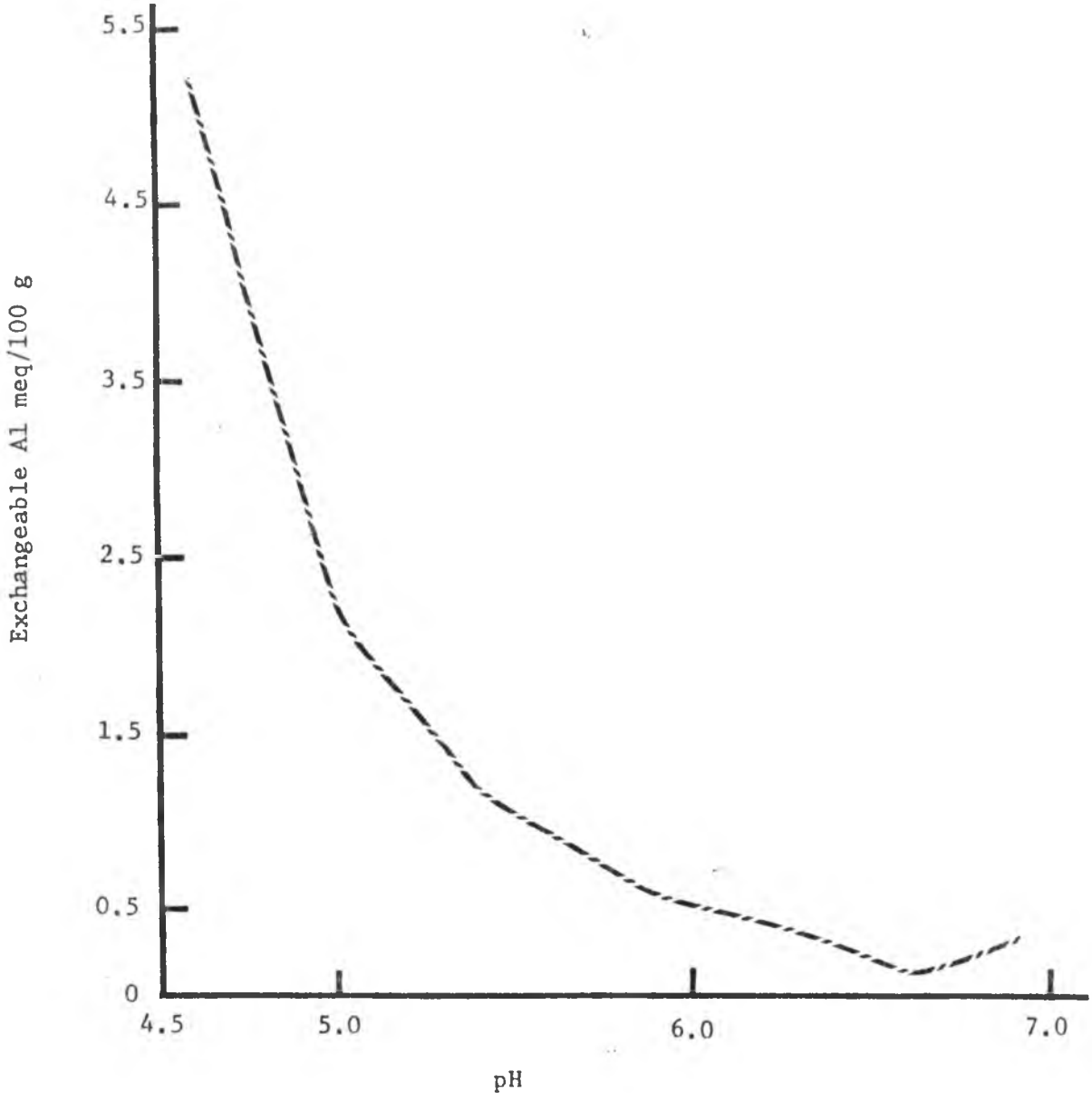


FIG. 15. EFFECT OF PH ON EXCHANGEABLE ALUMINUM IN PAUWELA SUBSOIL

SUMMARY

1. This study was designed to delineate the effects of calcium and pH on the growth and chemical composition of certain tropical and temperate legumes in a tropical subsoil containing high aluminum.

2. A gradient of applied calcium at various pH levels showed a significant effect of calcium but not of pH on subroot growth and calcium content. Subroot aluminum levels decreased with increasing calcium regardless of pH.

3. Adjustment of subsoil pH was accomplished with magnesium carbonate in the zero and low calcium treatments and levels of magnesium were equalized with magnesium sulfate. A depressing effect of the added magnesium on subroot growth was noted which was overcome by adding calcium.

4. With the zero calcium treatment at pH 4.8, it was impossible to differentiate both symptomatically and chemically that the low subroot growth was due to aluminum toxicity or calcium deficiency. However at pH 5.5 and 6.3 where exchangeable aluminum levels were decreased, depressed subroot growth was still apparent indicating that calcium was definitely deficient.

5. Application of calcium to the subsoil had no significant effect on subroot phosphorus in the calcium treated series. However the uptake was greater in the plus-Ca than in the zero-Ca treatment.

6. No significant difference in magnesium concentration or total magnesium uptake resulted from subsoil Ca application for all species except D. intortum.

7. On the basis of percentage Mg found in the subsoil tissue,

T. repens and S. gracilis are classed as high Mg-tolerant legumes (1.1% Mg), L. uliginosus as low Mg-tolerant (0.46% Mg) and D. intortum as intermediate (0.46-0.84%).

8. In the absence of subsoil Ca, an exchangeable Al value of 33 ppm or 1.2% Al saturation was found to be toxic for subroot growth of legumes in Pauwela subsoil.

9. The tropical legumes (S. gracilis and D. intortum) were superior to the temperate legumes (T. repens and L. uliginosus), on the basis of subroot dry weight yield.

APPENDIX TABLE I. ANALYSIS OF VARIANCE FOR T. REPENS GROWN 12 WEEKS IN SOIL CULTURE UNDER TWO LEVELS OF CA AND THREE PH VALUES

data - ave. of 3 reps.

Treatment	d.f.	Subsoil root dry weight M.S.	Concentration (ppm) P M.S.	Al M.S.	Ca M.S.	Mg M.S.	Uptake mg/pot P M.S.	Al M.S.	Ca M.S.	Mg M.S.
Ca	1	158.42	28,042	161,045	181,224	99413	.0002	.0085	.0068	.0058
pH	2	1467.76	984,478*	186,325	103,483	135779,309**	.0011	.0216	.0070	2.2172*
Ca x pH	2	1083.46	217,762	692,894	356,900	1638,747	.0029	.0017	.0076	.0773
Blocks	2	8095.60	1381,425	2620,638	201,885	6044,597	.0030	.0244	.0244	.5740
Error	10	2417.14	188,189	848,815	104,418	9435,169	.0051	.0202	.0098	.3962
Grand Mean		89.57	1460	1972	1668	11939	.128	.184	.169	1.179

*Significant at the 5% level.

**Significant at the 1% level.

APPENDIX TABLE II. ANALYSIS OF VARIANCE FOR L. ULIGINOSUS GROWN 12 WEEKS IN SOIL CULTURE UNDER THREE CA LEVELS AND THREE PH VALUES

data - ave. of 3 reps.

Treatment	d.f.	Concentration (ppm)					Uptake (mg/pot)			
		Subsoil root dry weight M.S.	P M.S.	Al M.S.	Ca M.S.	Mg M.S.	P M.S.	Al M.S.	Ca M.S.	Mg M.S.
Ca	2	49535.76**	4470,108	9029,061*	12525,343**	309,262	.094**	.162	.684**	.972*
pH	2	14950.43	1707,334	5669,764*	21,723	29008,669**	.045	.068	.188	.101
Ca x pH	4	6094.51	3261,830	3342,677	1494,078*	1683,505	.009	.033	.095	.129
Blocks	2	4930.78	822,569	16315,108	1683,865	1680,557	.001	.127	.082	.131
Error	16	5729.26	1045,157	1570,999	327,946	644,607	.014	.055	.092	.192
Grand Mean		106.50	1889	3248	2460	4673	.155	.252	.339	.465

*Significant at the 5% level.

**Significant at the 1% level.

APPENDIX TABLE III. ANALYSIS OF VARIANCE FOR S. GRACILIS GROWN 12 WEEKS IN SOIL CULTURE UNDER THREE CA LEVELS AND THREE PH VALUES

data - ave. of 3 reps.

Treatment	d.f.	Concentration (ppm)					Uptake (mg/pot)			
		Subsoil root dry weight M.S.	P M.S.	Al M.S.	Ca M.S.	Mg M.S.	P M.S.	Al M.S.	Ca M.S.	Mg M.S.
Ca	2	65835.05*	10807,436**	1392,406	2079,820*	12033,829	.055	.173	.223*	10.263*
pH	2	9173.19	2973,445**	341,589	271,627	18183,090	.023	.035	.047	2.523
Ca x pH	4	26300.76	4217,277**	135,373	234,872	4711,972	.036	.067	.083	5.028
Blocks	2	31323.14	1208,479	1765,482	1209,835	9874,506	.021	.057	.081	2.132
Error	16	15034.89	395,926	893,255	374,278	8224,625	.028	.055	.043	2.732
Grand Mean		134.95	2114	2052	1484	10702	.197	.223	.210	1.615

*Significant at the 5% level.

**Significant at the 1% level.

APPENDIX TABLE IV. ANALYSIS OF VARIANCE FOR D. INTORTUM GROWN 12 WEEKS IN SOIL CULTURE UNDER THREE CA LEVELS AND THREE PH VALUES

data - ave. of 3 reps.

Treatment	d.f.	Subsoil root dry weight M.S.	Concentration (ppm)				Uptake (mg/pot)			
			P M.S.	Al M.S.	Ca M.S.	Mg M.S.	P M.S.	Al M.S.	Ca M.S.	Mg M.S.
Ca	2	102404.85**	4001,966**	8020,552**	427,698*	16604,898*	.118**	.254*	.117**	6.854**
pH	2	3780.09	352,772	221,732	145,381	31697,066**	.011	.031	.007	.635
Ca x pH	4	6349.61	496,190	650,905	200,743	2818,497	.011	.034	.004	.674
Blocks	2	7626.54	1488,325	2191,744	140,967	4148,548	.020	.033	.018	1.065
Error	16	6894.12	343,543	1284,605	119,322	4040,707	.018	.052	.010	.822
Grand Mean		149.35	1431	2342	835	6362	.167	.289	.133	1.112

*Significant at the 5% level.

**Significant at the 1% level.

APPENDIX TABLE V. EFFECT OF SUBSOIL CA AND PH ON SUBSOIL ROOT AND TOP ROOT GROWTH OF FOUR LEGUME SPECIES

data - average of three replications

Species	pH H_2O 1:1 subsoil	Sub root (gm/pot)			Top root (gm/pot)			Sum (gm/pot)		
		no Ca subsoil	low Ca subsoil	high Ca subsoil	no Ca subsoil	low Ca subsoil	high Ca subsoil	no Ca subsoil	low Ca subsoil	high Ca subsoil
T. repens	4.8	.010	.065	.090	.300	.237	.218	.310	.302	.308
	5.5	-*	.116	.098	.307	.313	.289	.307	.429	.387
	6.3	-*	.097	.120	.395	.290	.253	.395	.387	.373
	mean	.010	.093	.102	.334	.280	.253	.337	.372	.356
L. uliginosus	4.8	.025	.224	.148	.316	.323	.261	.341	.547	.409
	5.5	.033	.200	.151	.379	.357	.382	.412	.557	.533
	6.3	.008	.053	.118	.246	.141	.227	.254	.194	.345
	mean	.022	.159	.139	.313	.273	.290	.335	.432	.429
S. gracilis	4.8	.045	.168	.086	.144	.143	.073	.189	.311	.159
	5.5	.050	.282	.152	.135	.159	.142	.185	.441	.294
	6.3	.014	.113	.306	.100	.119	.314	.114	.232	.620
	mean	.036	.181	.188	.126	.140	.176	.162	.328	.357
D. intortum	4.8	.046	.128	.251	.309	.268	.420	.355	.396	.671
	5.5	.018	.252	.248	.342	.307	.246	.361	.559	.494
	6.3	.019	.198	.185	.497	.317	.205	.516	.515	.390
	mean	.028	.193	.228	.382	.297	.290	.410	.490	.518

*No root growth.

APPENDIX TABLE VI. EFFECT OF SUBSOIL CA LEVELS AND PH VALUES ON SUM OF TOP ROOT AND SUBSOIL ROOT GROWTH AND ON TOP GROWTH OF FOUR LEGUME SPECIES

data - average of three replications

Species	Subsoil pH H_2O 1:1	Sum of root dry wt (gm/pot) in top and subsoil layers			Tops dry wt (gm/pot)		
		no Ca subsoil	low Ca subsoil	high Ca subsoil	no Ca subsoil	low Ca subsoil	high Ca subsoil
T. repens	4.8	.310	.302	.308	.687	.491	.640
	5.5	.307	.429	.387	.450	.760	.823
	6.3	.395	.387	.373	.725	.718	.753
	mean	.337	.372	.356	.620	.656	.738
L. uliginosus	4.8	.341	.547	.409	.661	.922	.814
	5.5	.412	.557	.533	.726	.884	.930
	6.3	.254	.194	.345	.429	.336	.568
	mean	.335	.432	.429	.605	.714	.770
S. gracilis	4.8	.189	.311	.159	.459	.698	.299
	5.5	.185	.441	.294	.547	.788	.567
	6.3	.114	.232	.620	.303	.508	1.307
	mean	.162	.328	.357	.436	.664	.791
D. intortum	4.8	.355	.396	.671	.548	.517	1.113
	5.5	.361	.559	.494	.569	.737	.681
	6.3	.516	.515	.390	.896	.658	.442
	mean	.410	.490	.518	.671	.637	.745

APPENDIX TABLE VII. EFFECTS OF SUBSOIL CA LEVELS AND PH VALUES ON NUTRIENT COMPOSITION AND UPTAKE OF T. REPENS AND L. ULIGINOSUS TOP SOIL ROOTS GROWN FOR 12 WEEKS

data - ave. of 3 reps.

Species and treatments meq Ca/100 g soil	Subsoil pH	Concentrations (%) in top soil roots			Uptake mg/pot in top soil roots		
		P	Al	Ca	P	Al	Ca
T. repens							
0.0	4.8	.169	.236	.284	.488	.648	.808
	5.5	.171	.233	.307	.476	.614	.886
	6.3	.136	.177	.286	.532	.612	1.112
4.5	4.8	.163	.255	.264	.323	.504	.712
	5.5	.143	.248	.256	.458	.781	.786
	6.3	.148	.212	.286	.428	.587	.805
11.0	4.8	.176	.189	.220	.316	.345	.442
	5.5	.154	.232	.308	.418	.608	.892
	6.3	.133	.205	.259	.334	.529	.657
L. uliginosus							
0.0	4.8	.181	.282	.389	.530	.630	1.136
	5.5	.138	.208	.384	.520	.689	1.453
	6.3	.282	.340	.541	.487	.612	1.235
4.5	4.8	.129	.155	.330	.415	.482	1.034
	5.5	.139	.247	.364	.463	.761	1.218
	6.3	.162	.311	.359	.240	.390	.506
11.0	4.8	.223	.204	.495	.523	.474	1.235
	5.5	.170	.194	.417	.673	.678	1.580
	6.3	.114	.269	.420	.239	.444	.942

APPENDIX TABLE VIII. EFFECTS OF SUBSOIL CA LEVELS AND PH VALUES ON NUTRIENT COMPOSITION AND UPTAKE OF D. INTORTUM AND S. GRACILIS TOP SOIL ROOTS GROWN FOR 12 WEEKS

data - ave. of 3 reps.

Species and treatments meq Ca/100 g soil	Subsoil pH	Concentrations (%) in top soil roots			Uptake mg/pot in top soil roots		
		P	Al	Ca	P	Al	Ca
D. intortum							
0.0	4.8	.126	.205	.258	.419	.620	.802
	5.5	.112	.170	.264	.384	.554	.937
	6.3	.122	.173	.285	.560	.663	1.181
4.5	4.8	.129	.189	.248	.380	.456	.697
	5.5	.125	.195	.228	.390	.495	.771
	6.3	.111	.185	.276	.357	.492	.895
11.0	4.8	.131	.146	.246	.574	.568	.984
	5.5	.150	.189	.293	.398	.372	.715
	6.3	.137	.221	.297	.280	.399	.619
S. gracilis							
0.0	4.8	.182	.266	.324	.303	.403	.492
	5.5	.224	.174	.390	.314	.213	.504
	6.3	.157	.251	.412	.169	.256	.417
4.5	4.8	.207	.190	.354	.299	.257	.493
	5.5	.144	.208	.378	.237	.290	.668
	6.3	.217	.202	.338	.312	.193	.429
11.0	4.8	.193	.204	.333	.141	.134	.222
	5.5	.207	.171	.383	.351	.244	.532
	6.3	.213	.176	.419	.522	.321	.908

APPENDIX TABLE IX. EFFECTS OF SUBSOIL CA LEVELS AND PH VALUES ON NUTRIENT COMPOSITION AND UPTAKE OF TOPS OF T. REPENS AND L. ULIGINOSUS GROWN FOR 12 WEEKS

data - ave. of 3 reps.

Species and treatments meq Ca/100 g soil		Subsoil pH	Concentrations (%) in tops				Uptake mg/pot in tops			
			P	Al	Ca	Mg	P	Al	Ca	Mg
T. repens	0.0	4.8	.170	.021	1.795	.457	.744	.122	12.67	2.66
		5.5	.171	.022	2.001	.273	.526	.083	10.78	1.18
		6.3	.136	.017	1.750	.245	.656	.095	12.33	1.90
	4.5	4.8	.162	.043	1.981	.586	.436	.146	10.68	2.84
		5.5	.142	.012	1.895	.835	.746	.089	13.94	6.69
		6.3	.148	.022	1.713	.648	.719	.145	13.07	4.88
	11.0	4.8	.176	.025	1.836	.753	.676	.088	11.33	4.05
		5.5	.154	.015	2.067	.584	.766	.106	17.83	5.00
		6.3	.133	.013	1.687	.481	.627	.091	12.65	3.65
L. uliginosus	0.0	4.8	.181	.014	1.173	.688	.731	.055	7.24	4.21
		5.5	.138	.012	1.266	.401	.726	.067	8.83	2.14
		6.3	.282	.025	1.712	.382	.426	.069	7.92	1.62
	4.5	4.8	.130	.010	1.114	.832	.795	.086	10.55	7.42
		5.5	.139	.014	1.302	.622	.764	.095	11.18	4.78
		6.3	.162	.017	1.187	.602	.400	.054	3.84	2.05
	11.0	4.8	.223	.009	1.348	.733	1.094	.055	11.00	6.06
		5.5	.170	.010	1.540	.586	1.169	.101	12.78	5.56
		6.3	.114	.019	1.960	.662	.426	.075	10.84	3.33

APPENDIX TABLE X. EFFECTS OF SUBSOIL CA LEVELS AND PH VALUES ON NUTRIENT COMPOSITION AND UPTAKE OF TOPS OF *D. INTORTUM* AND *S. GRACILIS* GROWN FOR 12 WEEKS

data - ave. of 3 reps.

Species and treatments meq Ca/100 g soil		Subsoil pH	Concentrations (%) in tops				Uptake mg/pot in tops			
			P	Al	Ca	Mg	P	Al	Ca	Mg
D. intortum	0.0	4.8	.126	.006	1.528	.327	.531	.031	8.22	1.43
		5.5	.112	.004	1.497	.234	.485	.019	8.70	1.35
		6.3	.122	.004	1.522	.183	.731	.029	15.23	1.67
	4.5	4.8	.129	.007	1.272	.709	.486	.034	6.22	3.68
		5.5	.125	.009	1.431	.594	.683	.043	11.53	3.65
		6.3	.111	.006	1.169	.584	.512	.035	7.65	3.50
	11.0	4.8	.131	.006	1.445	.598	1.500	.069	14.86	5.92
		5.5	.149	.006	1.651	.674	.928	.028	12.15	4.38
		6.3	.137	.007	1.493	.529	.283	.028	6.64	2.29
S. gracilis	0.0	4.8	.182	.016	1.830	.373	.685	.070	8.81	1.43
		5.5	.224	.012	2.072	.458	1.139	.062	9.41	3.00
		6.3	.157	.022	1.801	.284	.325	.043	5.65	0.95
	4.5	4.8	.207	.017	2.200	.593	.794	.077	14.53	3.99
		5.5	.154	.013	1.211	.462	.709	.064	12.93	3.62
		6.3	.217	.014	2.081	.656	1.521	.041	12.58	3.33
	11.0	4.8	.193	.021	2.123	.600	.338	.043	6.06	1.94
		5.5	.207	.014	2.080	.558	1.295	.061	11.16	3.78
		6.3	.213	.015	2.503	.313	2.438	.093	27.42	4.02

APPENDIX TABLE XI. EFFECTS OF CA AND PH LEVELS ON EXCHANGEABLE AL IN PAUWELA SUBSOIL AT THE END OF THE EXPERIMENT. ALL THE TREATMENTS HAD A BASAL DOSE OF 11 ME MG/100 G SOIL (MG IS SUPPLIED AS MG SO_4)

*data - ave. of 3 reps.

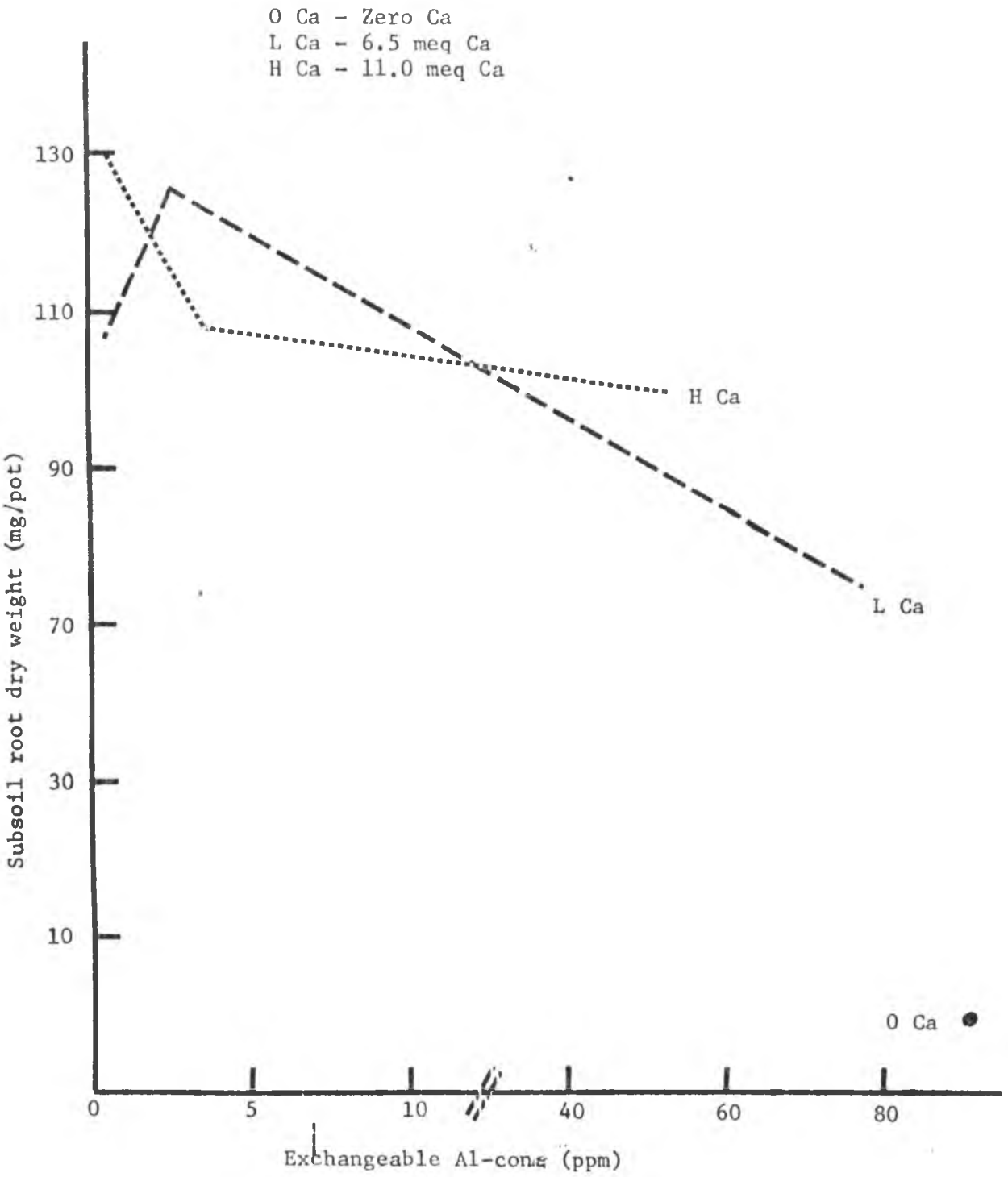
Species and treatments	Exchangeable Al (ppm)			mean
	pH 4.8	pH 5.5	pH 6.3	
T. repens				
0 Ca	94	5	0	33
4.5 Ca	78	3	1	27
11.0 Ca	47	3	1	17
Mean	73	4	1	
L. uliginosus				
0 Ca	92	2	1	32
4.5 Ca	77	8	3	29
11.0 Ca	43	4	1	16
Mean	71	5	2	
S. gracilis				
0 Ca	97	3	0	33
4.5 Ca	72	3	1	25
11.0 Ca	42	3	0	15
Mean	70	3	0	
D. intortum				
0 Ca	90	3	0	31
4.5 Ca	62	3	1	22
11.0 Ca	43	2	0	15
Mean	65	3	0	

Comments on Appendix Figures 1-4

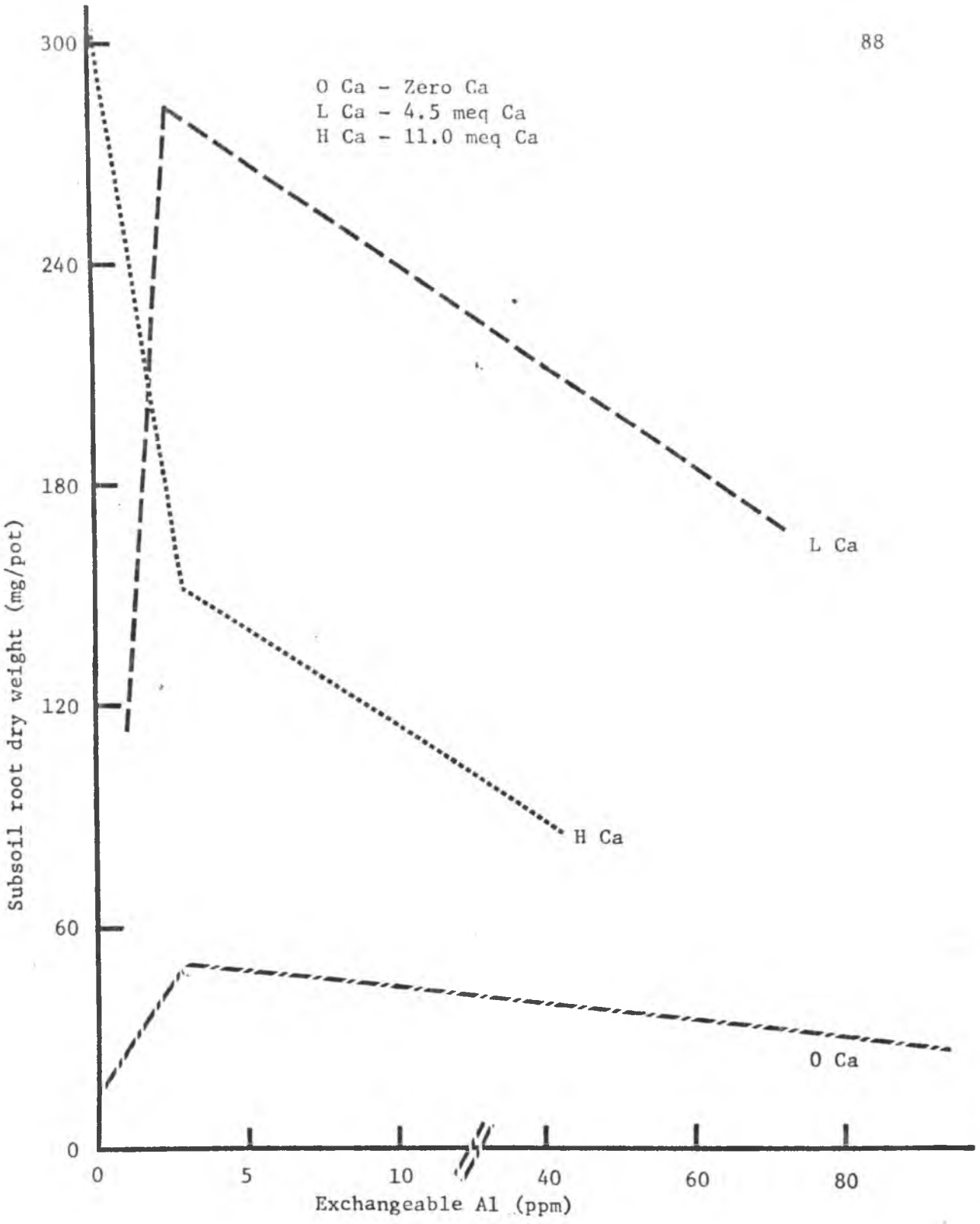
A fairly consistent drop in subsoil root dry weight yield accompanied the high pH treatments where the exchangeable aluminum in the subsoil was low. The following appendix figures are therefore included to illustrate this.

The following explanations may apply.

1. The complementary ion effect in which Al competes for exchange sites with Mg and thus allow Ca to be more available -- this would result in higher yields in the treatments with some Al in the system and lower yields in treatment with zero Al (which is the high pH treatments). At this high pH Mg is able to more successfully compete with Ca and possibly K, thus causing them to be limiting. Increased solubility of Al reduces this competition.
2. Possible micro-nutrient deficiencies due to the "high" pH.
3. A decrease in P uptake by the plant in the absence of Al.

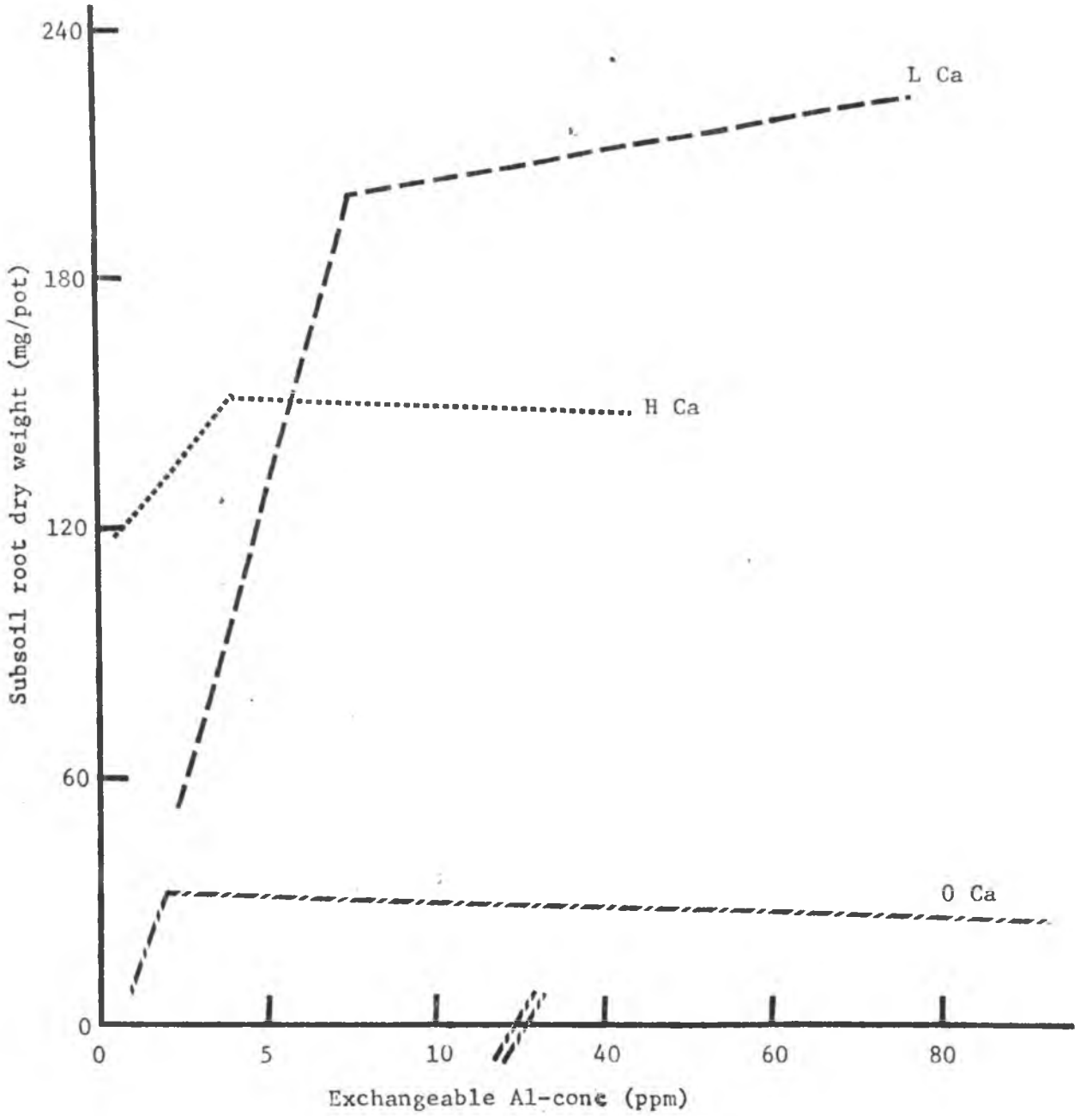


APPENDIX FIG. 1. EFFECTS OF CA LEVELS AND EXCHANGEABLE AL ON SUBSOIL ROOT DRY WEIGHT (MG/POT) OF T. REPENS GROWN 12 WEEKS IN PAUWELA SUBSOIL

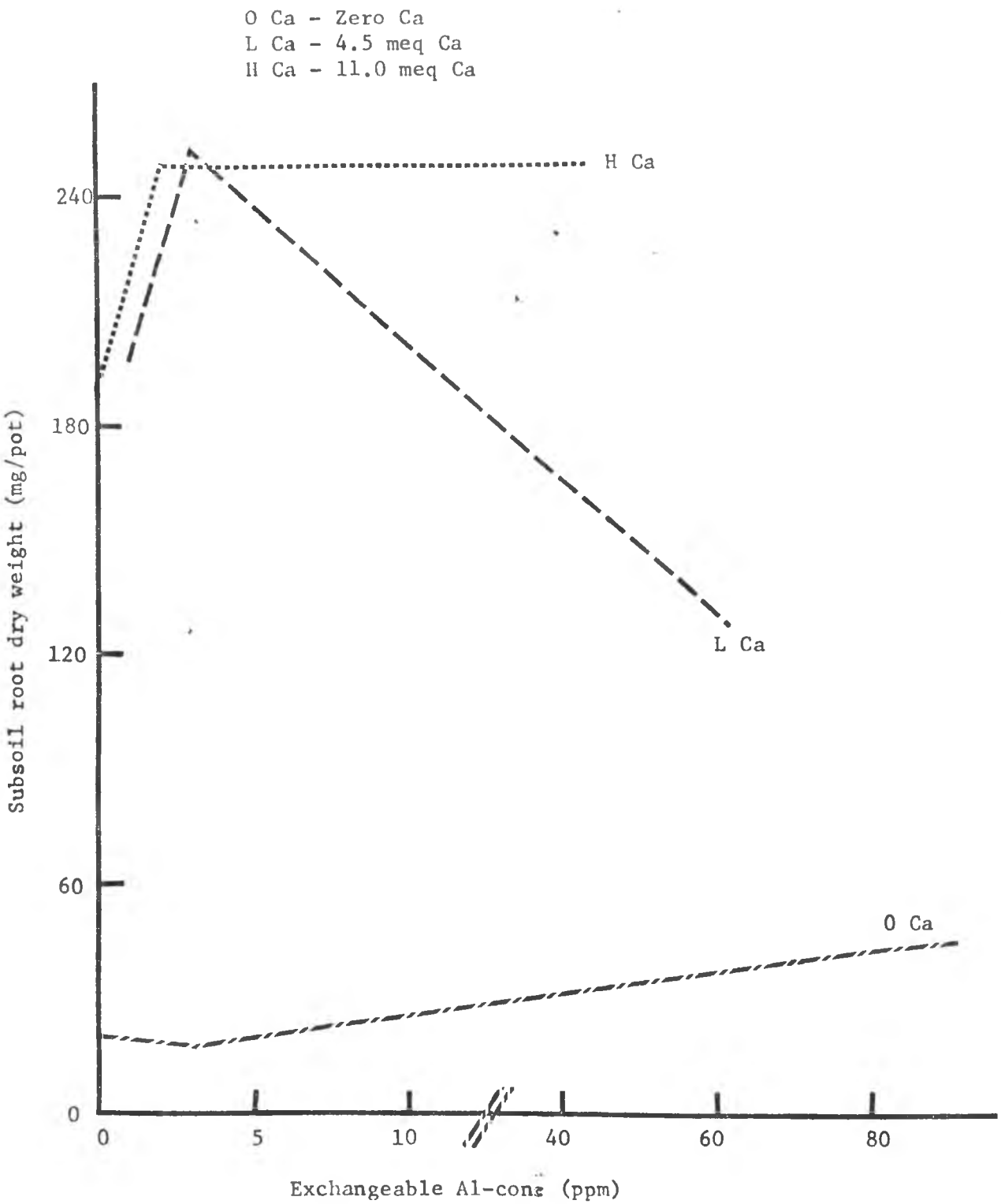


APPENDIX FIG. 2. EFFECTS OF CA LEVELS AND EXCHANGEABLE AL ON SUBSOIL ROOT DRY WEIGHT (MG/POT) OF S. GRACILIS GROWN 12 WEEKS IN PAUWELA SUBSOIL

O Ca - Zero Ca
L Ca - 4.5 meq Ca
H Ca - 11.0 meq Ca



APPENDIX FIG. 3. EFFECTS OF CA LEVELS AND EXCHANGEABLE AL ON SUBSOIL ROOT DRY WEIGHT OF L. ULIGINOSUS GROWN 12 WEEKS IN PAUWELA SUBSOIL



APPENDIX FIG. 4. EFFECTS OF CA LEVELS AND EXCHANGEABLE AL ON SUBSOIL ROOT DRY WEIGHT (MG/POT) OF D. INTORTUM GROWN 12 WEEKS IN PAUWELA SUBSOIL

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